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EDITED FOR

The American Physiological Society

BY

H. P. BOWDITCH, M.D., BOSTON
R. H. CHITTENDEN, PH.D., NEW HAVEN
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FREDERIC S. LEE, PH.D., NEW YORK
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THE

American Journal of Physiology.

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ON THE EXCRETION OF KYNURENIC ACID.[1]

BY LAFAYETTE B. MENDEL AND HOLMES C. JACKSON, PH.B.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

ALTHOUGH it is nearly half a century since Liebig discovered kynurenic acid in the urine of the dog, and this compound has long been assigned the constitution of an oxyquinoline-carboxylic acid,[2] there is much investigation yet demanded regarding its antecedents and origin in the metabolic processes of the body. The occurrence of kynurenic acid in the animal organism is interesting, because with the exception of *a*-methylquinoline recently isolated by Aldrich and Jones[3] from the anal secretion of Mephitis mephitica (common American skunk), it is, so far as we recall, the only quinoline compound discovered in connection with the animal body. Furthermore, the study of kynurenic acid production is important, because of the light which it promises to throw upon the transformations going on in the system, upon the constitution of the proteids from which the compound is derived, and possibly upon the physiological behavior of compounds like many of the alkaloids related to quinoline derivatives.

The early investigations on kynurenic acid can scarcely demand detailed consideration at present, since in the absence of satisfactory analytical methods the separation of uric acid (and possibly other substances) from the acid investigated was not accomplished.[1] It cannot be assumed that kynurenic acid completely replaces uric acid in the urine of the dog, inasmuch as the experiments of Solomin[2] have shown that both acids may occur together under appropriate conditions, and our own experience leads to a similar conclusion. Solomin found that although the uric acid nitrogen (determined by the Ludwig-Salkowski method) forms only a very small fraction of the total nitrogen excreted, the quantity of uric acid estimated per kilo of body weight may be as large as 0.01 gram, which corresponds with the average uric acid output per kilo in man. In the case of dogs in nitrogenous equilibrium, numerous experiments in this laboratory have given a considerably smaller excretion (0.003–0.004 gram per kilo).[3] The higher figures obtained by Solomin are perhaps attributable to the rather large quantities of proteid fed.[4]

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[1] A preliminary account of some of the experiments described in this paper was presented at a meeting of the American Physiological Society, December 28, 1897.

[2] SCHMIEDEBERG and SCHULTZEN: Ann. Chem. Pharm., 1872, clxiv, p. 155. KRETSCHY: Berichte d. deutsch. chem. Gesell., 1879, xii, p. 1673; Monatshefte für Chemie, 1881, ii, p. 57.

[3] ALDRICH and JONES: Journal of experimental medicine, 1897, ii, p. 439.

Regarding the immediate origin of kynurenic acid, little of a positive character is to be found in physiological literature. Its close relation to the diet, and its ready production after the ingestion of meat, have frequently been pointed out. Thus Schmidt[5] believed to have found kynurenic acid excretion to be greatest after feeding meat, and least with a bread diet, a milk diet yielding intermediate results. His figures for the various dietaries are, however, by no means comparable, since the quantities of the typical foodstuffs ingested in the three periods were not at all equivalent.[6] The experiments of Schmidt and of Rosenhain,[7] planned to observe the possible production of kynurenic acid from various quinoline derivatives[1] introduced directly into the organism, gave only negative results. The observations of Rosenhain and of Haagen,[2] in which a decrease of from thirty to fifty per cent of kynurenic acid was obtained after administration of intestinal antiseptics (*e. g.* salol, thymol, naphthalin) suggested a connection between intestinal putrefaction and kynurenic acid excretion. There is, however, no satisfactory evidence in these experiments that kynurenic acid has its origin in the decomposition going on in the intestine, since no data are given regarding the direct action of the drugs administered upon the food utilization and body metabolism. Thus it seems quite possible in view of our experiments that the diminished kynurenic acid excretion observed after naphthalin administration, for example, is to be attributed to poorer absorption of the proteid fed. It may also be recalled in this connection how many drugs, *e. g.*, antipyrin, antifebrin, salicylates, alcohol, exert a direct influence upon the production of uric acid; and accordingly similar specific effects may have been at work in Haagen's experiments. Again, Baumann[3] observed an undiminished excretion of kynurenic acid in a dog in which several days' starving and repeated doses of calomel had freed the intestine from putrefactive processes as shown by the absence of ethereal sulphates in the urine; while Haagen failed to find any decrease in kynurenic acid excretion after administering large doses of iodoform, which exerts a pronounced action upon the putrefactive processes in the intestine.[4] In this connection we may point out that Nuttall and Thierfelder[5] have lately demonstrated the possible origin of aromatic oxyacids in tissue metabolism, since they have found them in the urine of animals which were entirely free from all bacteria. It seems desirable to emphasize the preceding facts because the experiments of Haagen have repeatedly been misinterpreted and quoted in evidence of the intestinal origin of kynurenic acid,[6] although Haagen has carefully avoided such an interpretation of his observations.[1] Finally, the experiments of Capaldi[2] have given additional evidence against the assumed intestinal origin of kynurenic acid.

---

[1] Cf. for example, VOIT and RIEDERER: Zeitschrift für Biologie, 1865, i, p. 315.

[2] SOLOMIN: Zeitschr. f. physiol. Chemie, 1897, xiii, p. 497.

[3] Cf. CHITTENDEN: Journal of physiology, 1891, xii, p. 220. CHITTENDEN and GIES: This journal, 1898, i, p. 1.

[4] The daily diet of the 9-kilo dog consisted of meat, 400 grams; milk, 250 c.c.; and NaCl, 10 grams (*loc. cit.* p. 498). Regarding the increase in uric acid excretion following the ingestion of proteid, cf. SCHULTZE: Arch. f. d. ges. Physiol., 1889, xlv, p. 401; HERTER and SMITH: New York medical journal, June 4, 1892; HOPKINS: Schaefer's Physiology, 1898, i, p. 594.

[5] SCHMIDT: Ueber das Verhalten einiger Chinolinderivate im Thierkörper mit Rücksicht auf die Bildung von Kynurensäure. Inaugural-Dissertation. Königsberg, 1884. (Jaffé's laboratory.)

[6] The dog was fed, (*a*) meat, 1 kilo, (*b*) milk, 2 litres, (*c*) bread, 1 pound, respectively, per day.

[7] ROSENHAIN: Beiträge zur Kenntniss der Kynurensäurebildung im Thierkörper. Inaugural-Dissertation, Königsberg, 1886. (Jaffé's laboratory.) Cf. also, R. COHN: Zeitschr. f. physiol. Chemie, 1895, xx, p. 210.

---

[1] *E.g.* carbostyril, quinaldin, oxymethyl-quinoline, kynurin, antipyrin.

[2] HAAGEN: Ueber den Einfluss der Darmfäulniss auf die Enstehung der Kynurensäure beim Hunde. Inaugural-Dissertation, Königsberg, 1887. (Jaffé's laboratory.)

[3] BAUMANN: Zeitschr. f. physiol. Chemie, 1886, x, p. 131.

[4] Cf. MORAX: Zeitschr. f. physiol. Chemie, 1886, x, p. 321.

[5] NUTTALL and THIERFELDER: Zeitschr. f. physiol. Chemie, 1895, xxi, p. 109; 1896, xxii, p. 62.

[6] Cf. for example, HAUSER: Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 3; also, NEUMEISTER: Lehrbuch der physiol. Chemie, 2te Auflage,

In considering the immediate antecedents of kynurenic acid tyrosin is at once suggested. The behavior of this aromatic compound with reference to its possible synthesis to oxyquinoline-carboxylic acid in the body has been investigated by Hauser[3] and Solomin,[4] both of whom failed to obtain evidence of any direct relationship between the two substances.

Plan of present investigation. — The present investigation is an attempt to ascertain something more definite regarding the conditions which determine and modify kynurenic acid production and excretion. Unless otherwise stated, the data have been obtained with dogs. The animals were kept in suitable roomy cages which permit the separate collection of urine and fæces, and stand in a light, well-ventilated space. It was not found necessary to resort to catheterization, since the periods of observation always extended over more than one day and the animals soon became accustomed to discharge their urine with considerable regularity. The nitrogen was determined in the urine and diet by the Kjeldahl method; sugar, when present, was estimated by titration with Fehling's or Purdy's solution,[5] and kynurenic acid was found by the method of Capaldi,[6] which has proved very satisfactory. The product thus obtained always responded to Jaffé's test[7] and was crystalline; in a few urines a very small quantity of an amorphous substance was precipitated,

---

1897, p. 721. "Eine ältere, von Baumann stammende Angabe, dass die Quantität der Kynurensäure von den Fäulnissprocessen im Darm unabhängig sei, scheint durch die neueren Untersuchungen widerlegt zu sein."

[1] Cf. HAAGEN: *loc cit.*, p. 26, ". . . so ist es zweifelhaft, ob die nach anderen Antisepticis, besonders nach Naphthalin gefundene Verminderung der Kynurensäure auf Beschränkung der Darmfäulniss, oder ob sie nicht vielmehr auf anderen Umständen beruht."

[2] CAPALDI: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 87.

[3] HAUSER: Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 1.

[4] SOLOMIN: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 497.

[5] Cf. J. BISHOP TINGLE: American chemical journal, 1898, xx, p. 126.

[6] CAPALDI: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 92. Solomin (*ibid.* p. 498 note) recovered by this method 99 per cent of 0.210 gram kynurenic acid added to urine. The following figures show average duplicates obtained by us from a dog's urine containing small quantities: (*a*) 0.0852 gram, (*b*) 0.0872 gram.

[7] JAFFÉ: Zeitschr. f. physiol. Chemie, 1883, vii, p. 399.

which failed to give the characteristic reaction. As an immediate test for kynurenic acid — in the urine — the bromine water reaction, first recommended by Baumann,[1] was frequently found useful. Bromine, as is well known, usually gives an insoluble yellow precipitate when added to dog's urine, the composition of the precipitate depending upon the presence of phenol bodies, indol, or kynurenic acid. With a little experience it becomes easy to make use of the reaction in judging the relative amounts of kynurenic acid, since the latter ordinarily composes (as tetrabromkynurin[2]) by far the greater part of the precipitate formed.

Experiments on dogs. — For these experiments commercial cracker-dust containing as an average 1.46 per cent nitrogen was obtained in large quantity and kept in glass-stoppered bottles. This constituted the *carbohydrate* food fed. The *fat* used was a good quality of lard practically free from nitrogen. The other foodstuffs used will be referred to in the protocols.

The following experiments demonstrate the formation of kynurenic acid after the ingestion of various proteids of both vegetable and animal origin. The "dog biscuit" used was a commercial preparation containing dried meat, carbohydrates (sugar-beet), etc.; the albumin was commercial albumen e sanguine; the vegetable proteid was crystallized edestin (phytovitellin) prepared from hemp seed after the manner already described by one of us;[3] the Witte's "pepton" was the widely used product made up almost entirely of proteoses (from fibrin). The latter preparation contained 14 per cent N. A mixture of inorganic salts as recommended by J. Munk[4] was daily added to the diet in experiment C.

Various investigators have demonstrated that the proteoses and peptones may show caloric and nutritive values equivalent to those of the proteids from which they originate.[5] Several of our experiments (B, C, D) show a characteristic excretion of kynurenic acid after repeated feeding of proteoses (Witte's "pepton"). No disturbances of the gastro-intestinal tract (as with "Somatose," p. 9) were observed with this product.

---

[1] BAUMANN: Zeitschr. f. physiol. Chemie, 1877, i, p. 62.

[2] BRIEGER: Zeitschr. f. physiol. Chemie, 1881, iv, p. 89.

[3] CHITTENDEN and MENDEL: Journal of physiology, 1894, xvii, p. 49.

[4] J. MUNK: Virchow's Arch. f. d. exper. Pathologie, cxxxii, p. 102.

[5] Cf. MUNK AND EWALD: Die Ernährung, 1895, p. 34.

DOG A.

Date.	Body Weight.	Urine.				Food.
		Vol.	Sp. Gr.	Reaction.	Kynurenic Acid.	
1897.	kilos	c.c.		litmus	grams	grams
Nov. 22	9.0	110	1026	Acid	trace	carbohyd., 50; fat, 50; water, 200.
23	8.9	165	1006	"	none	" " "
24	8.8	160	1011	"	"	" " "
25	8.8	195	1013	"	"	" " "
26	8.7	175	1016	"	"	" " "
27	8.7	190	1027	Alkaline	0.012	**casein,**[1] 50; carbohyd., 50; fat, 40; water, 200.
28	8.8	230	1012	Acid	none	**casein,**[2] " " " "
29	8.7	125	1013	"	"	**casein,**[2] " " " "
30	8.9	195	1024	"	0.035	**casein,** " " " "
Dec. 1	8.9	165	1014	"	none	**casein,**[2] 100 " " "
2	8.9	250	1015	"	0.014	**casein,**[2] " " " "
3	8.8	70	1037	"	0 036	**meat,** 300; (little fat).
4	8.9	190	1046	"	0.124	" 400; " "
5	8.9	275	1045	"	0.103	" " " "

Dec. 6	8.9	205	1048	Acid	0.178	**meat**, 400; (little fat).
7	9.0	110	1047	"	0.058	carbohyd., 50; fat, 50; water, (200).
8	8.9	60	1041	"	none	" " "
9	8.9	90	1017	"	"	" " "
10	8.9	160	1017	"	"	**casein**, 150; carbohyd., 25; fat, 12; water, 75.
11	8.9	320	1021	"	0.023	" 240; " 50; " 25; " 100.
12	9.0	400	1017	"	0.057	" 300; " 50; " 50; " 200.
13	9.0	160	1018	"	0.032	" 75; " 25; " 25; " 100.
14	9.1	420	1018	"	0.121	" 350; " 50; " 50; " 200.
15	9.1	65	1030	"	0.017	carbohyd., 50; fat, 50; water, (100).
16	9.1	85	1020	"	0.005	" " "
17	9.1	120	1018	"	none	none (water).
18	8.9	85	1033	"	"	" "
19	8.8	70	1025	"	"	" "
20	8.6	65	1025	"	"	" "
21	8.7	50	1027	"	"	" "

---

[1] The casein in this and following experiments was either a commercial preparation, or casein prepared from milk, reprecipitated once and fed moist. The moist casein contained 25 to 30 per cent dry substance.

[2] Moist casein.

DOG B.

DATE.	BODY WEIGHT.	URINE.				FOOD.
		Vol.	Sp. Gr.	Reaction.	Kynurenic Acid	
1897.	kilos	c.c.		litmus	grams	grams
Dec 2	10.0	330	1012	Acid	none	none (water).
3	10.0	none	....	....	....	" "
4	9.9	185	1024	"	0.152	meat, 300; little fat.
5	9.8	400	1044	"	0.119	meat, 400; little fat.
6	9.5	43	1048	"	0.025	liver, 150.
7	9.6	240	1048	"	0.345	none (water).
8	...	none	....	....	0.093[1]	" "
9	9.4	175	1039	Acid	0.093	" "
10	9.0	none	....	....	0.097[1]	" "
11	...	"	....	....	0.097	" "
12	8.5	250	1034	"	0.097	" "
13	8.6	85	1034	"	0.081	carbohyd., 50; fat, 50; water, 200.
14	8.7	60	1048	"	none	" 100; " 100; " 300.
15	8.7	225	1016	"	"	" 75; " 75; " 150.

Dec. 16	8.6	115	1024	Alkaline	none	carbohyd., 25; fat, 25; water. "**Somatose**," 25.[2]
17	8.6	225	?	Acid	"	carbohyd., 30; fat, 25; water. "**Somatose**," 50.
18	8.4	230	?	Alkaline	"	carbohyd., 30; fat, 25; water. "**Somatose**," 50.
19	8.4	65	1024	Acid	"	"**Somatose**," 14, etc.
20	8.5	95	1024	"	"	carbohyd., 25; water, 50.
21	8.5	100	1028	"	"	" 125; " 200.

---

[1] The figures given for the daily kynurenic acid output of this period are calculated from the total amount excreted, there being no urine voided on part of the days.

[2] "Somatose" is a commercial product prepared by digestion of meat and composed largely of proteoses (70 to 80 per cent) with small quantities of peptone (R. H. CHITTENDEN: Dietetic and hygienic gazette, x, p. 47). The sample used contained 13.9 per cent N. In this animal it produced vomiting and diarrhœa, and doubtless only a portion of the material fed was absorbed. Irritation of the gastro-intestinal tract by large quantities of similar digestion products has frequently been observed. Cf. MUNK u. EWALD: Die Ernährung des gesunden und kranken Menschen, 1895, p. 427; also TREUPEL: Münchner med. Wochenschr., 1898, p. 611.

DOG C.

DATE.	BODY WEIGHT.	URINE.					FOOD.
1898.	kilos	Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic Acid.	grams
		c.c.		litmus	grams		
Mar. 21	9.9	179	1063	Acid	....	0.010	**dog biscuit.**
22	9.9	222	1058	"	....	0.036	"
23	9.9	none	....	"	....	....	"
24	10.1	380	1038	"	12.08	0.011	"
25	10.0	460	1029	Alkaline	8.59	0.099	**albumin**, 50; carbohyd., 75; fat, 40.
26	10.0	455	1037	Acid	11.68	0.168	" 75; " "
27	10.0	325	1040	"	8.53	0.187	" 100; " "
28	10.0	400	1035	"	10.27	0.106	" 100; " "
29	9.9	174	1048	"	5.60	0.053	" 50; " "
30	10.0	430	1029	"	13.42	0.125	" 50; " "
31	10.0	554	1029	"	17.94	0.141	**edestin**, 75; " "
April 1	9.9	385	1029	"	13.08	0.137	" 100; " "
2	10.1	415	1022	"	9.32	0.191	" 100; " "
3	10.1	350	1018	"	6.69	0.009	**albumin**, 15; " "
4	10.0	222	1050	"	9.61	0.018	" 15; " "
5	10.0	276	1030	Alkaline	8.17	0.428	**Witte's "pepton,"** 75; carbohyd., 100; fat, 40.

DOG D.

DATE.	BODY WEIGHT.	URINE.				FOOD.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Kynurenic Acid. grams	grams
April 17	8.3	202	1027	Acid	0.028	**albumin**, 15; carbohyd., 50; fat, 40; water.
18	8.3	105	1020	"	trace	" " " "
19	8.3	180	1023	"	none	" " " "
20	8.3	150	1030	"	0.017	" " " "
21	8.3	188	1041	"	0.333	**albumin**, 15; carbohyd., 50; fat, 40; **Witte's "pepton,"** 75; water; daily.
22	8.4	312	1050	"	0.276	
23	8.3	143	1055	Alkaline	0.182	
24	8.6	550	1015	Neutral	trace	**albumin**, 15; carbohyd., 50; fat, 40; water.
25	8.5	264	1014	Alkaline	none	" " " "
26	8.4	258	1019	Acid	"	" " " "

DOG E

DATE.	BODY WEIGHT.	URINE.					FOOD.
		Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic Acid.	
1898.	kilos	c.c.		litmus	grams		grams
May 20	9.9	366	1014	Acid	5.02	0.044	**albumin**, 15; carbohyd., 50; fat, 40; water.[1]
21	9.9	195	1014	Neutral	2.05	0.039	" " " "
22	9.9	510	1011	Alkaline	3.57	0.041	" " " "
23	9.8	500	1009	Neutral	2.77	none	" " " "
24	9.8	820	1016	Alkaline	5.02	0.175	" 80; " " "
25	9.8	665	1016	"	8.05	0.149	" " " "
26	9.8	650	1017	"	6.83	0.200	" " " "
27	9.8	340	1012	"	2.98	0.055	" 15; " " "
28	9.8	455	1010	"	2.32	0.028	" " " "
29	9.7	410	1023	"	7.48	0.064	" 40; **gelatin**, 40; carbohyd., 50; fat, 40; water.
30	9.5	440	1017	"	1.47	none	" " " " "
31	9.5	560	1023	"	12.17	0.108	" " " " "
June 1	9.4	335	1011	"	2.73	0.080	" 15; " " " "
2	9.5	410	1015	"	4.00	0.027	" 15; " " " "
3	9.4	480	1017	"	7.77	none	**gelatin**, 80; carbohyd., 50; fat, 40; water.
4	9.3	435	1019	"	7.74	"	" " " "
5	9.3	240	1024	"	5.99	"	" " " "
6	9.4	560	1009	"	3.19	"	**albumin**, 15; " " "
7	9.5	350	1010	"	1.84	"	" 15; " " "
8	9.5	425	1014	"	2.70	0.049	" 40; " " "
9	9.5	750	1010	"	3.39	0.167	" 80; " " "

[1] Ad libitum.

The results obtained in the preceding experiment are summarized in the following table, which gives the daily averages for various feeding periods.

DOG E.—SUMMARY.

(Giving daily averages for various feeding periods.)

DATE.	URINE.		FOOD.	
1898.	Nitrogen.	Kynurenic Acid.	Nitrogen.	Proteid.
	grams			
May 21-23	2.79	0.027	1.75	albumin, 15.
24-26	6.63	0.175	9.36	" 80.
27, 28	2.65	0.044	1.75	" 15.
29-31	7.04	0.057	10.20	albumin, 40; gelatin, 40.
June 1, 2	3.36	0.053	1.75	" 15.
3-5	7.17	none	11.04	gelatin, 80.
6, 7	2.51	none	1.75	albumin, 15.

In view of the peculiar chemical and physiological behavior of gelatin in contrast to the ordinary proteids, some experiments were undertaken with this albuminoid (Dogs F, G, H). Commercial gelatin, containing 13.8 per cent N, was fed, it being eagerly eaten when mixed with water and the other food stuffs as indicated. Occasionally 1 to 2 grams of Liebig's extract of beef were added to improve the flavor, while a mixture of inorganic salts as recommended by J. Munk[1] was daily given with the food. The following dog F, which had sometime previously been used in a phlorhizin experiment, had been fed very large quantities of casein on the days immediately preceding the experiment.

At the conclusion of the experiment on the dog H, the animal was starved for eighteen days. Body-weight fell from 7.6 kilos to 6.0 kilos. During this period 1220 c.c. of urine, containing 107 mgr. kynurenic acid, were eliminated.

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[1] J. MUNK: Virchow's Arch. f. d. exper. Pathologie, cxxxii, p. 102.

DOG F.

DATE.	BODY WEIGHT.	URINE.					FOOD.[1]
		Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic Acid.	
1898.	kilos	c.c.		litmus	grams		grams
Feb. 1	7.2	138	1043	Acid	6.11	0.073	**casein**, 25; fat, 50; carbohyd., 50.
2	7.2	77	1032	“	2.52	0.028	“ “ “
3	7.2	248	1015	“	3.51	none	“ “ “
4	7.1	112	1030	“	3.79	“	**gelatin**, 35.
5	7.2	230	1027	“	7.15	“	“ 40.
6	7.2	156	1028	“	5.65	“	“ 45.
7	7.0	112	1022	Alkaline	....	0.008	**albumin**, 20; carbohyd., 35.
8	7.0	155	1026	“	....	0.046	“ 30; “
9	7.0	228	1025	“	3.72	0.060	“ 40; “
10	7.0	477	1018	“	7.23	0.104	“ 50; “
11	7.1	260	1033	“	3.26	0.138	“ 60; “ 10.
12	7.0	248	1050	“	10.16	0.076	“ 60; **gelatin**, 35; carbohyd., 10.
13	6.9	198	1049	“	8.02	0.003	“ “ “
14	6.9	174	1044	“	9.57	0.021	“ 50; “ 20; “ 35.
15	6.9	480	1028	Alkaline	10.15	0.123	“ 60; “ “
16	6.9	338	1020	“	9.71	0.072	“ 40; fat, 40; “ 50.
17	6.9	294	1017	“	....	none	“ 30; “ 30; “
18	6.9	258	1026	“	....	0.079	“ ‘ “ “
19	7.0	455	1014	Neutral	....	none	“ 25; “ 40; “
20	7.0	275	1016	“	....	“	“ “ “ “
21	7.0	298	1013	Alkaline	....	“	“ “ “ “

[1] Water was given ad libitum.

DOG G.

DATE.	BODY WEIGHT.	URINE.					FOOD.
		Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic Acid.	
1898.	kilos	c.c.		litmus	grams	grams	grams
Mar. 14	8.6	137	1046	Acid	....	0.085	none (water).
15	8.3	none	....	"	....	[1] 0.069	" "
16	8.3	150	1043	"	....	0.069	" "
17	8.0	70	1048	"	....	0.069	" "
18	8.0	211	1035	"	....	0.137	**albumin**, 20; carbohyd., 50; fat, 40; water.
19	8.0	170	1023	Neutral	....	0.023	" " " "
20	8.0	320	1012	Acid	....	0.028	" " " "
21	8.0	383	1015	"	....	0.050	" " " "
22	8.0	214	1021	"	....	0.068	" " " "
23	8.0	412	1017	"	....	0.075	" " 75; " "
24	8.0	345	1012	"	2.47	0.016	" 15; " " "
25	8.0	200	1018	"	2.07	0.025	" " " "
26	8.0	146	1020	"	1.79	0.028	" " " "
27	8.0	293	1021	"	3.55	0.024	" " " "
28	8.0	275	1017	"	3.13	0.011	" " " "
29	8.0	184	1054	"	8.16	none	" 15; **gelatin**, 75; carbohyd., 75; fat, 40; water.
30	8.1	258	1041	"	10.69	"	" " 100; " " "
31	8.2	624	1030	"	19.66	"	" " " " "
Apr. 1	8.0	548	1026	"	12.15	0.015	" 50; " 50; " " "
2	8.1	290	1038	"	5.96	0.053	**albumin**, 100; carbohyd., 75; fat, 40; water.
3	8.0	265	1043	"	7.61	0.091	" " " "
4	8.1	288	1018	"	3.52	none	carbohyd., 75; fat, 40; water.
5	8.1	164	....	"	1.67	"	" "

[1] The figures given for the daily Kynurenic acid output of this period are calculated from the total amount excreted, there being no urine voided on one of the days.

DOG H.

DATE.	BODY WEIGHT.	URINE.					FOOD.
		Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic Acid.	
1898.	kilos	c.c.		litmus	grams		grams
Mar. 13	8.5	41	1033	Acid	1.18	none	none; water.
14	8.3	37	1031	"	1.08	"	" "
15	8.1	57		"	1.81	0.012	" "
16	8.0	41	1033	"	0.94	none	" "
17	7.9	none	....	....	1.35	0.015	" "
18	7.8	none	....	....	[1] 1.35	[1] 0.015	" "
19	7.8	60	1050	"	1.35	0.015	" "
20	7.6	160	1013	"	1.87	0.010	" "
21	7.5	128	1019	"	2.13	0.004	casein,[3] 100; fat, 100; water, 75.
22	7.6	105	1017	"	1.53	0.017	" 50; " "
23	7.6	94	1017	"	1.56	0.016	" " "
24	7.6	105	1023	"	2.32	0.026	" " "
25	7.7	136	1020	"	2.46	0.018	" " "
26	7.8	56	1031	"	1.47	0.011	" 75; " "

Mar. 27	7.8	116	1023	Acid	2.39	0.041	casein,[3] 75; fat, 100; water, 75.
28	7.8	300	?	"	....	none	" 75; gelatin, 35; fat, 100; water.
29	7.6	325	?	"	8.01	"	" " " "
30	7.6	290	?	Alkaline	....[2]	"	" 35; " 70; " "
31	7.6	270	1030	Acid	6.85	"	" " 70; " "
April 1	7.7	162	1031	"	5.53	0.061	" 350.
2	7.6	230	1035	"	8.68	0.137	" 375.
3	7.6	330	1024	"	8.81	0.028	" 350.
4	7.7	174	1044	"	9.11	0.017	" 175; gelatin, 30.
5	7.6	150	1047	"	8.27	none	" 90; " 45.
6	7.6	75	1040	"	3.97	"	fat, 100; " 15.
7	7.6	204	1022	"	....	0.013	casein,[3] 215; carbohyd., 50; fat, 25; gelatin, 25.
8	7.7	295	1023	"	....	0.034	" 300; gelatin, 30.

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[1] Cf. note 1, Dog G.

[2] A small quantity of fæces became mixed with the urine and made an accurate urine-nitrogen determination impossible.

[3] Moist casein.

DOG H.

DATE.	BODY WEIGHT.	URINE.					FOOD.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	grams
Mar. 13	8.5	41	1033	Acid	1.18	none	none; water.
14	8.3	37	1031	"	1.08	"	" "
15	8.1	57		"	1.81	0.012	" "
16	8.0	41	1033	"	0.94	none	" "
17	7.9	none	....	....	1.35	0.015	" "
18	7.8	none	....	....	[1] 1.35	[1] 0.015	" "
19	7.8	60	1050	"	1.35	0.015	" "
20	7.6	160	1013	"	1.87	0.010	" "
21	7.5	128	1019	"	2.13	0.004	casein,[3] 100; fat, 100; water, 75.
22	7.6	105	1017	"	1.53	0.017	" 50; " "
23	7.6	94	1017	"	1.56	0.016	" " "
24	7.6	105	1023	"	2.32	0.026	" " "
25	7.7	136	1020	"	2.46	0.018	" " "
26	7.8	56	1031	"	1.47	0.011	" 75; " "

Mar. 27	7.8	116	1023	Acid	2.39	0.041	**casein,**[3] 75; fat, 100; water, 75.
28	7.8	300	?	"	....	none	" 75; **gelatin,** 35; fat, 100; water.
29	7.6	325	?	"	8.01	"	" " " "
30	7.6	290	?	Alkaline	....[2]	"	" 35; " 70; " "
31	7.6	270	1030	Acid	6.85	"	" " 70; " "
April 1	7.7	162	1031	"	5.53	0.061	" 350.
2	7.6	230	1035	"	8.68	0.137	" 375.
3	7.6	330	1024	"	8.81	0.028	" 350.
4	7.7	174	1044	"	9.11	0.017	" 175; **gelatin,** 30.
5	7.6	150	1047	"	8.27	none	" 90; " 45.
6	7.6	75	1040	"	3.97	"	fat, 100; " 15.
7	7.6	204	1022	"	....	0.013	**casein,**[3] 215; carbohyd., 50; fat, 25; **gelatin,** 25.
8	7.7	295	1023	"	....	0.034	" 300; **gelatin,** 30.

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[1] Cf. note 1, Dog G.

[2] A small quantity of fæces became mixed with the urine and made an accurate urine-nitrogen determination impossible.

[3] Moist casein.

Discussion of the preceding analytical data. — So far as has been observed, all aromatic compounds found in the organism of the higher animals are derived either from benzene derivatives introduced into the system, or from the proteids, which must accordingly contain aromatic radicals. A synthesis of the latter in the animal body from carbohydrates or fats scarcely seems probable in view of the accumulated experimental data bearing on the problem.[1] The present study of kynurenic acid excretion lends additional force to this view as applied to quinoline derivatives. Thus kynurenic acid is almost always found in the urine during starvation, a condition in which body proteids form the source of the nitrogenous compounds excreted. This observation, repeatedly made (*e. g.* Dogs G, H, J, K), leaves little doubt that kynurenic acid is a true product of proteid katabolism, and for the most part, at least, is not dependent for its origin on the putrefactive processes in the intestine, — a possibility which has already been mentioned.

Quantitatively considered, kynurenic acid production bears a more or less direct relation to the variations in the decomposition of proteid material, whether the latter be the "tissue proteid" of a starving animal, or introduced as food and the kynurenic acid formed incidental to its metamorphosis. Moreover, our experiments furnish repeated evidence that kynurenic acid is a concomitant or direct product of accelerated proteid metabolism. There is no lack of evidence that the proteid molecule may break down in the body into a nitrogenous and non-nitrogenous portion. The former part is doubtless rapidly further broken down, oxidized and synthesized perhaps into various nitrogenous constituents of the urine. The non-nitrogenous moiety is less speedily eliminated. It may be converted into glycogen, or dextrose, or fat; and forming, as it does, the major part of the original molecule, it may be distributed to the tissues more slowly in proportion as they demand it.[2] The experiments of Feder[3] and of Reilly, Nolan and Lusk[4] indicate that most of the nitrogen of ingested meat is, on the other hand, eliminated within a few hours. It may be imagined, then, that kynurenic acid is one of the nitrogenous products derived from this rapidly eliminated nitrogenous radical of the original proteid; and it scarcely seems unreasonable to assume that this acid represents the metabolic end-product of quinoline-yielding radicals in the molecule.[1] In this connection it will be remembered that R. Cohn[2] was able to obtain a pyridine derivative as a decomposition product of casein, thus demonstrating the possible presence of the pyridine ring in the constitution of the proteid molecule.

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[1] Cf. BAUMANN: Zeitschr. f. physiol. Chemie, 1886, x, p. 123.

[2] Cf. REILLY, NOLAN and LUSK: This journal, 1898, i, p. 404.

[3] FEDER: Zeitschrift für Biologie, 1881, xvii, p. 541.

[4] REILLY, NOLAN and LUSK: *loc. cit.*, p. 404 flg.

Granting conditions like those referred to, several probabilities are indicated. For example, it should follow that the extent of proteid katabolism going on should influence very largely the production of kynurenic acid. Our experiments with non-nitrogenous diet (fat and carbohydrate) are quite in harmony with this. Excessive proteid decomposition is avoided under such conditions, and kynurenic acid rapidly disappears from the urine. (Cf. Dog A, B.) Equally suggestive are the results obtained with a mixed diet containing only small amounts of proteid. The proteid-sparing effect of the other foodstuffs is here well brought out. In one animal (Dog A) an absence of kynurenic acid excretion during the first seven days of starvation was noted. The observation was an exceptional one, but perhaps not without significance, since the animal had an unusually well nourished appearance and the "fat" condition was remarkably persistent even during the later days of starvation. We are inclined to attribute the results to the rather exceptional condition of the animal, since it is well known that the extent of nitrogenous katabolism in inanition is modified in a pronounced way by the relative as well as absolute amount of body fat in the individual.[3]

Kynurenic acid excretion has also been investigated in three dogs which were kept in nitrogenous equilibrium on a fixed diet of meat, fat, and carbohydrates. The animals were used in a research carried out in this laboratory on the influence of borax and boric acid on nutrition. Through the kindness of Dr. Gies we have obtained equivalent portions of each day's urine and have examined them for kynurenic acid, the fractions of each period being united and analyzed collectively. Further data are taken from the tables already published.[4]

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[1] Cf. also KRETSCHY: Monatshefte für Chemie, 1881, ii, p. 85.

[2] R. COHN: Zeitschr. f. physiol. Chemie, 1896, xxii, p. 171.

[3] Cf. MUNK and EWALD: Die Ernährung, 1895, pp. 22–23.

[4] CHITTENDEN and GIES: This journal, 1898, i, p. 1.

FIRST EXPERIMENT.

Period.	Nitrogen balance.	Uric acid.	Kynurenic acid.
(9 days).	grams		
Fore	−0.981	0.428	none
Borax (5 grams)	−1.928	0.411	0.101
After	−0.117	0.389	none

SECOND EXPERIMENT.

Period.	Nitrogen balance.	Uric acid.	Kynurenic acid.
(10 days).	grams		
Fore	+0.118	0.504	none
Boric acid (1–2 grams) . .	−1.506	0.386	trace
After	−0.980	0.508	none

THIRD EXPERIMENT.

Period.	Nitrogen balance.	Uric acid.	Kynurenic acid.
(8 days).	grams		
Normal	+0.429	0.315	none
Borax (2–5 grams) . . .	−0.801	0.293	none
After	+0.661	0.248	none
Boric acid (1–3 grams) . .	+2.174	0.310	none
After	+2.122	0.354	none
Borax (8 grams)	**−4.878**	**0.295**	**0.414**
After	−0.661	0.328	none

It will be observed from the tables that the only periods during which kynurenic acid appeared in the urine were those in which administration of borax or boric acid gave rise to a direct stimulation of proteid metabolism. This is particularly brought out in the third experiment, during which a daily dose of eight grams of borax produced a nitrogen deficit of over four grams. Uric acid, however, was continually present in the urine of the dogs employed; and the results obtained are in harmony with the opinion that kynurenic acid excretion is a phenomenon accompanying pronounced stimulation of proteid katabolism rather than ordinary conditions of body equilibrium.

The gelatin experiments. — Eckhard[1] stated that after feeding gelatin to a dog he failed to find kynurenic acid in the urine. Rosenhain[2] likewise obtained no kynurenic acid after feeding two and a half pounds of gelatin and six pounds of bread to a large dog in the course of a week. After a meal of one kilo of meat, however, the same animal excreted as much as 0.995 gram of kynurenic acid during the succeeding twenty-four hours. No data are presented to show that the gelatin was absorbed satisfactorily.

The results obtained with gelatin feeding in the present investigation afford an interesting confirmation of previous observations. The experiments of J. Munk[3] have shown that in dogs over two-thirds of the required proteid of the diet may be replaced by gelatin with maintenance of nitrogenous equilibrium. Gelatin is as a rule readily digested and burned in the body, and has a pronounced proteid-sparing action like a typical non-proteid food. Lusk and his co-workers have recently shown that gelatin — like the proteids proper — may yield sixty per cent of sugar in the metabolic changes it undergoes in the body, as was evidenced by the amount of sugar excreted when the albuminoid was fed to a fasting animal in phlorhizin diabetes. Gelatin differs chemically from the ordinary proteids in that tyrosin has not been found among its decomposition products.[4] The absence of tyrosin is suggestive of a deficiency in aro-

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[1] ECKHARD: Ann. Chem. Pharm., 1856, xcvii, p. 358.

[2] ROSENHAIN: Beiträge zur Kenntniss der Kynurensäurebildung im Thierkörper. Inaugural-Dissertation. Königsberg, 1886, p. 8.

[3] J. MUNK: Arch. f. d. ges. Physiol., 1894, lviii, p. 309.

[4] HALLIBURTON: Schaefer's Physiology, 1898, i, p. 71. It may be added that K. B. Lehmann failed to accomplish proteid synthesis in rats by feeding them with gelatin and tyrosin (Sitzungsber. d. morphol.-physiol. Gesellsch. in München, 10 März, 1885). Contrary to the current statements, cf. NEUMEISTER: Lehrbuch der physiol. Chemie, 1897, p. 63, pure gelatin gives a reaction with Millon's reagent which may perhaps be due to a far smaller proportion of aromatic radicals than is present in ordinary proteids. See VAN NAME: Journal of experimental medicine, 1897, ii, p. 128.

DOG J.

DATE.	BODY WEIGHT.	URINE.						FOOD.
1898.	kilos	Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic acid.	Dextrose.	grams
		c.c.		litmus	grams			
Jan. 11	8.8	197	1029	Acid	3.90	0.023	....	none.
12	8.5	....	....	....	....	0.008[1]	....	"
13	8.3	96	1033	"	2.54	0.008[1]	....	"
14	8.1	120	1034	"	3.64	0.078	....	"
15	8.0	....	....	....	....	0.070[1]	....	" water.
16	7.9	142	1030	"	4.91	0.070[1]	....	" "
17	7.7	73	1044	"	2.64	0.060	....	" "
18	7.5	172	1056	"	4.57	0.130	10.49	**phlorhizin**, 1; (three times a day).
19	7.5	300	1058	"	8.18	0.168	26.67	" "
20	7.3	360	1056	"	7.88	0.141	25.03	" "
21	7.2	226	1063	"	6.27	0.150	19.06	**casein**, 100; fat, 100; water, 75.
22	7.0	176	1037	"	3.82	0.063	....	" 50; " "
23	6.9	98	1025	"	2.04	0.029	....	" "
24	6.9	112	1017	"	1.78	0.059	....	" " fat, 100; water, 75.

matic groups in the molecule, and it is not unlikely that this fact may account for the absence of the related compound kynurenic acid (oxyquinoline-carboxylic acid) when gelatin exclusively is fed. (Cf. Dogs E, F.) Out of 905 grams gelatin fed to small dogs in fourteen days we have obtained only 0.015 gram kynurenic acid, and that on a single day. Moreover, the nitrogen determinations in the urine (cf. Dogs E, F, G, H) give evidence of a ready absorption of the gelatin ingested; while the experiments including simultaneous feeding of typical proteids like

Jan. 25	6.9	134	1016	Acid	1.56	0.030	....	**casein**, 50; fat, 100; **water**, 75.
26	6.9	76	1018	"	1.22	0.006	....	" 75; " 150; " 100.
27	7.0	127	1021	"	2.11	none	....	" 25; " 50; " 100.
28	6.9	265	1023	"	3.48	0.082	....	" 320; water.
29	7.0	370	1016	"	6.95	0.091	....	" 545; "
30	7.1	295	1021	"	7.83	0.122	....	" 574; "
31	7.2	335	1026	"	12.24	0.139	....	" 515; "

DOG J.—SUMMARY.

PERIOD.	URINE.	
	Nitrogen	Kynurenic Acid.
	grams	
Fasting, 3 days	7.5	0.200
Phlorhizin, 3 days	20.63	0.440
Casein, etc., 3 days	12.13	0.243

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[1] Daily average calculated from two days' urine.

casein and albumin with the gelatin show that the latter exercises no *specific* action in preventing kynurenic acid excretion when sufficient proteid is given along with it. Indeed, the behavior of gelatin precisely resembles that of the carbohydrates and fats. These experiments considered in connection with Lusk's observations indicate that the essential physiological peculiarities of gelatin are to be sought in the chemical structure of the *nitrogenous* portion of the molecule; and the failure of the albuminoid to give rise to kynurenic acid in the dog is doubtless associated with the lack of certain aromatic radicals in its make-up. Lastly, attention is directed to the ready assimilation of crystallized vegetable proteid (Dog C), and to the evidence offered of the close physiological relationship between the animal and vegetable products, despite minor differences in chemical structure.[1]

Phlorhizin experiments. — In confirmation of the view that kynurenic acid excretion is incidental to excessive proteid katabolism, additional experiments on animals suffering from phlorhizin diabetes are presented. It has been shown that under these conditions sugar production goes on in the fasting animal directly at the expense of tissue proteid, the amount of nitrogen in the urine going parallel with the amount of sugar excreted;[2] and the sugar excretion may thus go on even in the absence of glycogen in the liver.[3] Lusk[4] and his co-workers have shown that in the fasting dog a constant ratio of dextrose to nitrogen excreted is maintained during phlorhizin diabetes (D: N:: 3.75: 1), and that feeding meat or gelatin does not change the ratio. The figures indicate a production of about 60 grams of dextrose from 100 grams of proteid, and it has furthermore been found that in this form of diabetes the proteid metabolism may increase to an extent as high as 560 per cent above that in simple inanition. Tables J and K show the extent of kynurenic acid production observed on two fasting dogs during phlorhizin diabetes. The phlorhizin, dissolved in dilute sodium carbonate solution, was introduced subcutaneously about every eight hours. Water was given ad libitum.

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[1] Cf. RUTGERS: Zeitschrift für Biologie, 1888, xxiv, p. 351.

[2] Cf. for example, CREMER u. RITTER: Zeitschrift für Biologie, 1893, xxix, p. 256.

[3] THIEL: Arch. f. exper. Pathol. and Pharmakol, 1887, xxiii, p. 142. Cf. also HÉDON: Comptes rendus de la soc. de biologie, Paris, 1897, (10), iv, p. 60.

[4] REILLY, NOLAN and LUSK: This journal, 1898, i, p. 395.

DOG K.

DATE.	BODY WEIGHT.	URINE.						FOOD.
1898.	kilos	Vol.	Sp. Gr.	Reaction.	Nitrogen.	Kynurenic Acid.	Dextrose.	grams
		c.c.		litmus	grams			
May 19	12.4	....	....	....	4.21	0.178	....	none; water.
20	12.3	244	1042	Acid	4.21	0.178	....	" "
21	12.2	....	....	....	2.42	0.065	....	" "
22	12.0	....	....	....	2.42	0.065	....	" "
23	11.9	....	....	....	2.42	0.065	....	" "
24	11.8	290	1046	Acid	2.42	0.065	....	" "
25	11.8	290	1058	"	9.00	0.208	39.15	**phlorhizin**, 1; three times a day.
26	11.6	420	1055	"	8.82	0.255	29.82	" 1; twice a day.
27	....	520	1048	"	10.86	0.342	35.88	none; water.
28	9.9	325	1051	"	7.35	0.172	22.42	" "
29	9.6	210	1050	"	....[1]	0.073	11.55	" "
30	9.4	265	?	"	4.38	0.053	12.98	" "
31	9.2	405	1025	"	2.47	none	....	" "
June 1	9.3	180	1020	"	....	none	....	" "

[1] Fæces in the urine.

Here again the production of kynurenic acid during fasting is observed. Coincident with the appearance of the sugar in the urine occurs a rise in nitrogen excretion accompanied likewise by an increase in kynurenic acid. The figures become more striking when the daily averages of the period preceding and succeeding the phlorhizin days are presented in contrast.

The increased output of kynurenic acid attending the large increase in proteid katabolism (over that in inanition) thus justifies the emphasis placed upon the close relationship between these factors. Owing to less careful conditions of experiment (lack of catheterization, etc.) the ratio of dextrose to nitrogen is somewhat lower than that found by Lusk. In the experiment on Dog K the ratio is higher. In this animal sugar persisted in the urine for several days after the phlorhizin injection. The ratio of dextrose to nitrogen during the phlorhizin days was as 3.68 : 1. The other phenomena observed resemble those of the preceding experiment, namely, increased kynurenic acid excretion accompanying stimulated proteid decomposition,[1] as shown in the curves of Fig. 1.

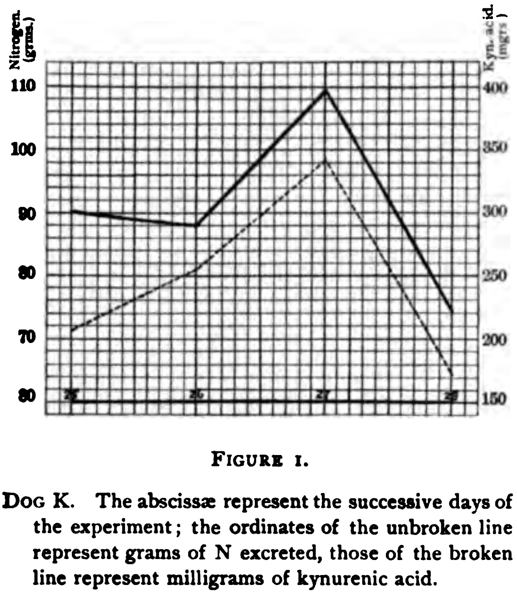

FIGURE I.

DOG K. The abscissæ represent the successive days of the experiment; the ordinates of the unbroken line represent grams of N excreted, those of the broken line represent milligrams of kynurenic acid.

Amyl nitrite experiment. — The production of diabetes in the dog by means of amyl nitrite has been demonstrated by a number of investigators.[2] We have also studied the action of this drug in the case of a dog. The 10-kilo animal had been fed three days on a diet consisting of albumin, 15 grams; carbohydrate, 50 grams; fat, 40 grams; — under which conditions nothing more than traces of kynurenic acid were found in the urine. On the fourth day the animal received three subcutaneous injections of amyl nitrite (about 6 c.c. being given in all). By the following day the dog was dead. The urine (128 c.c.) removed from the bladder contained no kynurenic acid; 4.69 grams sugar were present, and the ratio of dextrose to nitrogen was found as 7.4 : 1. Traces of albumin were also found, in agreement with observations of Araki.[1] The high ratio of dextrose to nitrogen indicates that the sugar found could not have its origin wholly in proteid decomposition, nor was this to be expected under these conditions (previous feeding, etc.). But Thiel[2] has shown that amyl nitrite fails to produce characteristic glycosuria in hens, although phlorhizin is effective in these animals. Amyl nitrite diabetes is therefore doubtless of a distinctly different type from that produced by phlorhizin; at any rate the absence of kynurenic acid in the urine in our single experiment corresponds with the slight evidence of proteid katabolism obtained.

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[1] In the case of one dog which excreted no kynurenic acid during six days' starvation we were unable to get similar results with phlorhizin.

[2] ARAKI: Zeitschr. f. physiol. Chemie, 1891, xv, p. 553. The older literature is referred to here.

Experiments on other animals. — So far as we are aware, no one has succeeded in finding kynurenic acid in the urine of any animal other than the dog. Hofmeister,[3] after careful examination, failed to find it in human urine. It has likewise been found missing in the urine of the rabbit,[4] wolf,[5] and fox.[5] We have searched for kynurenic acid in the urine of the cat during inanition as well as during meat and milk diet, but always with negative results. In view of the evidence of our experiments regarding the formation of kynurenic acid in the dog from body proteids during inanition, several rabbits were starved for varying periods (three to five days) and the urine then examined. No kynurenic acid was found. Lastly the urine of man has been examined in wasting diseases (severe diabetes) without avail. Since Hauser[6] and Solomin[7] have observed that kynurenic acid introduced into the organism of man, rabbit, or dog is in good part destroyed (especially in the case of the first two), it may be, as Solomin suggests, that kynurenic acid is absent from human and rabbit's urine not because it fails to be produced, but rather because it is destroyed in these organisms as rapidly as it is formed.

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[1] ARAKI: Zeitschr. f. physiol. Chemie, 1891, xv, p. 553. The older literature is referred to here.

[2] THIEL: Arch. f. exper. Pathol. u. Pharmakol., 1887, xxiii, p. 142.

[3] HOFMEISTER: Zeitschr. f. physiol. Chemie, 1881, v, p. 69. Eckhard, long before, had missed it in human urine; see Ann. Chem. Pharm., 1856, xcvii, p. 358.

[4] SCHMIDT: Ueber das Verhalten einiger Chinolinderivate im Thierkörper mit Rücksicht auf die Bildung von Kynurensäure. Inaugural-Dissertation, Königsberg, 1884.

[5] CAPALDI: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 87.

[6] HAUSER: Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 1.

[7] SOLOMIN: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 497.

Theoretical considerations regarding the quantity of kynurenic acid obtainable. — That the absolute amount of kynurenic acid produced at any time should be large is scarcely to be expected. If, as the experiments of Lusk and his co-workers have indicated, the proteid molecule yields on cleavage in the body an amount of sugar equal to nearly 60 per cent, there remains " a nitrogen-containing radical in which the carbon and nitrogen would appear in the atomic ratio of 2.2 of C to 1 of N."[1] Now kynurenic acid, $C_{10}H_7NO_3$, contains 10 of C to 1 of N; obviously the nitrogenous proteid radicals could not yield large quantities of a body of the composition indicated.

Summary. — Kynurenic acid is a direct product of proteid katabolism, and, as Baumann's experiments indicated, does not owe its immediate origin to putrefactive changes in the intestine.

Kynurenic acid excretion accompanies accelerated proteid decomposition, whether this condition be brought about by starvation, ingestion of large amounts of proteid food, or through the action of drugs (borax, phlorhizin).

Similar results follow the ingestion of both animal and vegetable proteids, as well as proteoses; gelatin, however, does not give rise to kynurenic acid in metabolism, acting precisely like the carbohydrates in this respect. In conditions of ordinary nitrogenous equilibrium or under the influence of proteid-sparing foods, kynurenic acid excretion is greatly diminished or absent.

The observations suggest the presence of quinoline-like radicals in the proteid molecule; the existence of a large carbohydrate group is also confirmed.

Uric acid and kynurenic acid may occur together in dog's urine, as Solomin has found. Kynurenic acid is absent from the urine of the cat during fasting and proteid feeding, and is not found in the urine of the rabbit during inanition.

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[1] REILLY, NOLAN and LUSK: This journal, 1898, i, p. 409.

ON THE MODIFICATION OF RIGOR MORTIS RESULTING FROM PREVIOUS FATIGUE OF THE MUSCLE, IN COLD-BLOODED ANIMALS.

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THE effect of previous fatigue upon the process of rigor mortis was first investigated by Brown-Séquard.[1] He stated that when one leg of a rabbit was removed immediately after death and stimulated with a constant current, it stiffened in ten minutes, whereas the other leg did not enter into rigor for five hours. Nagel[2] obtained a similar result in the isolated muscle of cold-blooded animals. He fatigued one gastrocnemius of a frog either through strychnia poisoning, or through electrical stimulation of the sciatic nerve for fifteen minutes. A record was taken by the graphic method of the process of rigor in both muscles, and it was found that the tetanized muscle entered into rigor about sixteen hours sooner than the normal muscle; also that the whole amount of shortening in the fatigued muscle was about one-third less than it was in the muscle which had not been fatigued.

Both Brown-Séquard and Nagel obtained their results in the course of experiments undertaken with the object of determining the influence of the central nervous system upon rigor mortis. They did not, apparently, consider the relation of the results they obtained to the question of the direct influence of the previous condition of the muscle. The present investigation was undertaken in order to determine whether the effect of fatigue upon rigor mortis is constant; and this point having been decided in the affirmative, the experiments were continued with the object of ascertaining, if possible, to what changes in the fatigued muscle itself the modification induced by fatigue is due.

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[1] BROWN-SÉQUARD: Gazette medicale de Paris, 1849, pp. 881, 999; quoted by M. BIERFREUND: Archiv. f. d. ges. Physiol., 1888, xliii, p. 211.

[2] NAGEL, W.: Experimentelle Unterschungen über die Todten-Starre bei Kaltblütern. Archiv. f. d. ges. Physiol., 1894, lviii, p. 279.

I. DESCRIPTION OF METHOD.

The experiments were conducted entirely upon frogs. Most of the observations were made upon heat rigor, from motives of convenience, although in order to establish beyond doubt that the results obtained in this way could justifiably be applied to the normal process a few experiments were performed in which rigor was allowed to come on in the usual manner. In every instance the results were recorded by the graphic method. The brain and spinal cord of the frog were first destroyed. After this the tendons of both gastrocnemii were freed beneath the skin, and both sciatics were exposed. The frog was now fastened on a board and a hook passed through one of the tendons by means of which the muscle was connected with a lever that recorded the curve of fatigue upon a revolving drum. This lever carried a weight that varied from five to fifteen grams, according to the size of the frog. The muscle was fatigued by stimulation of the sciatic nerve with an ordinary induction coil. Simple breaking shocks were thrown into the muscle at intervals of four seconds by means of a Baltzar drum. It was occasionally found desirable to increase this rate of stimulation when it could be done without inducing symptoms of tetanus. During the stimulation the secondary coil of the induction apparatus was at first placed as far from the primary coil as was possible consistently with the production of maximal contractions; but after symptoms of exhaustion appeared with the coil in this position it was brought gradually nearer until finally complete fatigue with discontinuous stimuli was obtained with the secondary coil over the primary. One Edison-Lalande cell was used in the primary circuit. The time required for complete fatigue varied from one to four and a half hours; during this period the nerve was kept covered with a tent of filter paper wet with normal saline solution, and was also moistened from time to time with the same liquid. When the muscle failed to give any further response to stimulation the frog was taken down and both gastrocnemii muscles removed, the kneejoint and a portion of the femur remaining attached to the muscle. Each gastrocnemius was now placed in an apparatus especially adapted for this experiment. It consisted of a cylinder about three and a half centimetres in diameter, within which was a second cylinder of the same length but much narrower. The lower end of the outer cylinder was closed by a cork, into the middle of which the inner cylinder fitted, and the space between the two was filled with water. A glass tube, open at one end and sealed at the other, was inserted by its open end into the cork with which the outer cylinder was closed. This tube was twenty centimetres in length, and was bent upon itself so that it projected from the apparatus at an obtuse angle. As the tube communicated by its open upper end with the water-filled compartment between the two cylinders, it was, of course, itself filled with water; and when a Bunsen burner was placed under the closed lower end the heat was transmitted by convection currents to the volume of water above. The inner cylinder mentioned before was closed at its lower end by a rubber stopper, through the middle of which passed a narrow glass tube, and its top was covered by a closely fitting hard rubber cover. When a few cubic centimetres of water were placed in the bottom of this cylinder it became a perfect moist chamber heated by the surrounding water; the temperature within it was recorded by a thermometer which passed through the cover. In this moist chamber the muscle was suspended, the femur to which it remained attached being screwed into a socket in the cover specially intended for this purpose. To the hook inserted in the tendon of the muscle was attached a thread that passed to the exterior through the glass tube in the lower end of the moist chamber. This string was connected with a light aluminium lever carrying a weight of one gram, an amount just sufficient to keep the lever steady. When the two muscles were securely in place, each in its own moist chamber, the two pens at the ends of the levers were adjusted so as to write one above another on a drum revolving at the rate of once an hour. With care and close attention it was found possible to elevate the temperature of the two moist chambers with very nearly the same rapidity. The application of heat by means of the Bunsen burner was continued not only up to the point at which rigor appeared, but afterwards, until the muscle gave no further sign of contraction.

II. EFFECT OF FATIGUE UPON RIGOR MORTIS.

When the two muscles, one of which had been fatigued, were heated the following results were obtained. The heat rigor appeared from 5° to 13° C. earlier in the fatigued muscle, the majority of cases showing a difference of not less than 10° C. The rigor was usually complete at nearly the same temperature in both muscles. The whole amount of shortening in the fatigued muscle was one half to one third less than in the normal muscle. These differences manifest themselves very plainly in a comparison of the tracings taken from the two muscles. An example is given in the accompanying illustration of curves plotted from one of the records.

The results of twelve experiments are given below in tabular form. Four others showed the same general characteristics, although the records were not sufficiently accurate for tabulation. It may be added that several experiments of the same kind performed by the writer in the physiological laboratory of Bryn Mawr College before the present investigation was begun, agree entirely with the later results.

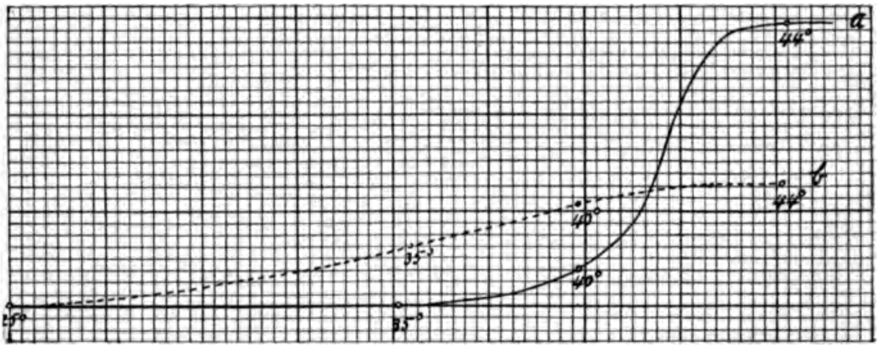

Figure 1. One half the original size. Curve of heat rigor in: *a*, the normal muscle; *b*, the fatigued muscle.

In order to determine whether the inferences drawn from the effect of fatigue upon heat rigor could justifiably be applied to rigor occurring in the usual manner, an experiment was performed in which after one gastrocnemius muscle was completely fatigued rigor was allowed to come on in both muscles at the temperature of the room, which was 20° to 23° C. In the normal muscle rigor began in about twenty-two hours, and was complete in about fifty-one hours; in the fatigued muscle it made its appearance in one hour and was complete in six hours. The whole amount of shortening in the fatigued muscle was one third less than in the other. This result coincides exactly with those obtained by the application of heat, and was confirmed by several other experiments of a similar character.

TABLE I.

Showing effect of previous fatigue on heat rigor.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.
I	Normal	38	43	29
	Fatigued	25	41	14
II	Normal	37	44	32
	Fatigued	25	35	11
III	Normal	37	45	29
	Fatigued	25	40	8
IV	Normal	37	43	35
	Fatigued	27	41	20
V	Normal	39	43	25
	Fatigued	34	40	10
VI	Normal	37	42	31
	Fatigued	28	40	10
VII	Normal	37	43	20
	Fatigued	25	40	14
VIII	Normal	38	44	21
	Fatigued	25	30	9
IX	Normal	40	43	28
	Fatigued	30	44	18
X	Normal	39	43	36
	Fatigued	33	40	14
XI	Normal	36	44	38
	Fatigued	27	43	17
XII	Normal	37	43	33
	Fatigued	29	40	19

III. INVESTIGATION INTO THE CAUSE OF THE EFFECT OF FATIGUE ON RIGOR MORTIS.

It having been shown that previous fatigue of the muscle undoubtedly hastened the appearance of rigor and also lessened the extent of shortening, the next step was to investigate the cause of this modification. The first idea that suggested itself was obviously that the removal of the products of fatigue by irrigation with a neutral solution would probably restore the normal rigor.

Irrigation with 0.5 per cent sodium chloride solution. — In this and similar experiments the unfatigued muscle was first excluded from the circulation by a ligature tied tightly around the thigh. The other muscle was then irrigated by placing a cannula in the aortic arch of one side and ligating all other branches. Thus the entire animal was irrigated with the exception of the head and lungs and the excluded leg. The success of the irrigation in the gastrocnemius muscle was usually indicated by the amount of œdema exhibited. From five hundred to a thousand cubic centimetres of liquid were used at a time.

Four experiments in which the fatigued leg was washed out with normal salt solution for a period of time varying from fifteen minutes to two hours showed no restoration of the normal rigor. Two other experiments in which Ringer's solution (NaCl, 0.7%, 100 c.c.; KCl, 1%, 3 c.c.; $CaCl_2$, 1%, 2.6 c.c.) was substituted for the saline solution, gave the same result. In one of the latter the method was slightly varied by fatiguing both legs and then irrigating one of them, the other fatigued leg serving in this case as a control.

TABLE II.

Irrigation after fatigue with solution of 0.5 per cent NaCl, or with Ringer's solution.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Normal	36	43	21	
	Fatigue + Irrigation .	30	40	9	
II	Normal	34	43	34	
	Fatigue + Irrigation .	29	45	26	
III	Normal	35	41	37	
	Fatigue + Irrigation .	26	39	23	
IV	Normal	37	44	58	
	Fatigue + Irrigation .	27	43	33	
V	Normal	40	42	59	Ringer's solution.
	Fatigue + Irrigation .	32	42	43	
VI	Fatigue	27	41	22	Ringer's solution. Both legs fatigued; one leg irrigated.
	Fatigue + Irrigation .	28	42	22	

Irrigation with sodium carbonate. — As the appearance of fatigue in worked muscle seems to be connected with the formation of acid products during contraction, it seemed desirable to ascertain whether the normal rigor could be restored in a fatigued muscle by irrigation with a feebly alkaline solution. The muscle was therefore washed out with normal saline solution containing 5 milligrams of Na_2CO_3 to 100 cubic centimetres of liquid. It may be said at once that these experiments proved the least satisfactory portion of this investigation. The difficulty of washing out the blood-vessels with an alkaline solution is very great, for the alkali has a strong tendency to constrict them, so much so that it was sometimes impossible to induce the circulating fluid to flow at all after the passage of 200 to 300 cubic centimetres, even under very high pressure. In all experiments of this nature, therefore, there exists some uncertainty as to whether the fatigued muscle was, or was not, completely washed out. In seven out of eight experiments in which the normal muscle was compared with the one which had been fatigued and irrigated, the curve of rigor in the fatigued muscle retained the characteristics of fatigue, although in one case — number two in the accompanying table — the amount of shortening was very nearly the same in both muscles. In the remaining experiment, number eight, there seems to be a partial restoration of the normal curve; but it is possible that in this case the apparent restoration may be the result of incomplete fatigue of the muscle. Two additional experiments are given in which both muscles were fatigued and one of them irrigated. In one of these, number nine, the irrigation had, practically, no results. In the other, number ten, the muscle was irrigated with Ringer's solution, to which was added the usual percentage of sodium carbonate. This was done in hopes that the potassium salts contained in it might by their relaxing effect upon the blood-vessels counteract the opposite influence of the alkali. This hypothesis proved correct, for eight hundred cubic centimetres of the solution were circulated through the leg without difficulty. The result showed a very slight increase in the amount of shortening in the irrigated muscle, but there was no other evidence of restoration, and the two curves were practically the same.

Irrigation with acids. — As the results obtained by irrigation with an alkali were not entirely conclusive, owing to the difficulty of irrigation, it was decided to attack the question from the other end, and ascertain whether washing out a normal muscle with an acid solution would induce the modification of rigor which occurs after fatigue. One leg was therefore ligated and the gastrocnemius muscle of the other side irrigated with sodium chloride solution containing

TABLE III.

Irrigation after fatigue with normal saline solution containing 0.05 per cent Na_2CO_3.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.
I	Normal	35	42	39
	Fatigue + Irrigation .	26	41	29
II	Normal	37	44	30
	Fatigue + Irrigation .	28	42	28
III	Normal	38	42	39
	Fatigue + Irrigation .	28	42	27
IV	Normal	32	44	27
	Fatigue + Irrigation .	28	40	6
V	Normal	37	42	50
	Fatigue + Irrigation .	25	40	32
VI	Normal	29	40	59
	Fatigue + Irrigation .	26	36	13
VII	Normal	37	43	54
	Fatigue + Irrigation .	26	42	27
VIII	Normal	38	43	27
	Fatigue + Irrigation .	35	42	22
IX	Fatigue	25	42	24
	Fatigue + Irrigation .	27	42	25
X	Fatigue	28	42	23
	Fatigue + Irrigation .	28	43	29

0.025 per cent lactic acid. Two experiments of this kind showed that rigor in the irrigated muscle not only did not set in earlier than in the other muscle, but that its appearance was actually delayed. There was no difference in the amount of shortening in the two muscles. Two experiments in which acetic acid was substituted for lactic gave the same result. As these four experiments are quite positive it seems justifiable to conclude that the effect of fatigue on rigor is not due to the influence of increased acid production during contraction.

Irrigation with carbon dioxide. — One experiment in which the muscle was irrigated for thirty minutes with saline solution saturated with carbon dioxide gave absolutely negative results.

TABLE IV.

Irrigation of normal muscle with normal saline solution containing acid.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Normal	36	43	27	Lactic acid 0.025 %.
	Irrigated	39	45	26	
II	Normal	33	44	26	" " "
	Irrigated	38	44	26	
III	Normal	36	43	17	Acetic acid 0.025 %.
	Irrigated	39	44	16	
IV	Normal	36	43	21	Acetic acid 0.01 %.
	Irrigated	41	44	20	

Irrigation with sodium oxalate. — In order to ascertain whether the variation in heat rigor was due to a possible variation in the calcium salts during fatigue, the muscle was irrigated with saline solution containing 0.4 per cent of sodium oxalate. Three experiments of this kind showed that the rigor of the irrigated muscle was wholly unaffected, although the precipitation of the calcium salts caused its indirect irritability to disappear completely.

TABLE V.

Irrigation with normal saline solution containing 0.4 per cent sodium oxalate.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Irritability after Irrigation.
I	Normal	37	44	54	Normal.
	Irrigated	38	44	57	Lost.
II	Normal	32	42	47	Normal.
	Irrigated	31	42	43	Lost.
III	Normal	38	43	48	Normal.
	Irrigated	38	43	47	Lost.

Irrigation, with extract of fatigued muscle. — It seemed possible that the modification of rigor present in fatigued muscle might be due to the accumulation of waste products other than carbon dioxide or lactic acid. In order to investigate this question the following experiment was made. The lower extremities of two very large frogs were completely fatigued; all the muscle tissue was at once removed and extracted with seventy-five cubic centimetres of normal saline solution at 45° C. for about thirty minutes. A medium sized frog was placed completely under the influence of curare, after which a ligature was tied around the right leg, and the left leg irrigated with the saline extract at short intervals for about half an hour. This method is precisely similar in its details to that given by Ranke in his work on *Tetanus*, and the passage of the fluid through the blood-vessels was accompanied by all the symptoms which Ranke describes, namely, general twitching of the muscles, loss of direct irritability in them, and arrest of the heart-beat. At the end of irrigation the direct irritability of both muscles was carefully tested, and found to be almost completely abolished in the irrigated muscle, although it remained normal in the other. It was confidently expected that after such indications of the effect of the irrigation upon the whole organism some modification of rigor would ensue; on the contrary, it was found that the heat rigor pursued its normal course in both muscles, the only difference being that the amount of shortening in the irrigated muscle was somewhat less than in the other. Two other experiments of a similar nature gave like results, but in both the amount of contraction was greatest in the case of the irrigated muscle. It would seem, therefore, that the variation in this respect may be considered accidental, the character of the two curves being the same in all cases. In one additional experiment an alcoholic extract of fatigued muscle was substituted for the saline extract previously used. This alcoholic extract was prepared by cutting up the fatigued muscles very fine, grinding them in a mortar, and then placing them in five times their own amount of 95 per cent alcohol. After three days the alcohol was filtered off and evaporated to dryness; the residue was then extracted at the boiling point with 200 cubic centimetres of normal saline solution and filtered. The reaction of this extract as well as that made with saline solution was distinctly, though not strongly, acid. The results of this experiment were precisely similar to those just described, except that the symptoms accompanying the passage of the alcoholic extract through the blood-vessels were somewhat less severe than during the circulation of the saline extract.

TABLE VI.

Irrigation with extract of fatigued muscle.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Normal	38	43	64	Saline extract.
	Irrigated	38	44	50	
II	Normal	35	43	37	" "
	Irrigated	35	43	44	
III	Normal	36	43	31	" "
	Irrigated	35	42	35	
IV	Normal	39	42	55	Alcoholic extract.
	Irrigated	38	42	55	

Irrigation with dextrose. — As it seemed clearly established that the changes in rigor mortis after fatigue could not be explained by any one of the hypotheses that at first seemed reasonable, the idea now suggested itself that possibly the using up of the glycogen which is known to occur when muscle is severely worked might be responsible for the alteration in the rigor. An effort was therefore

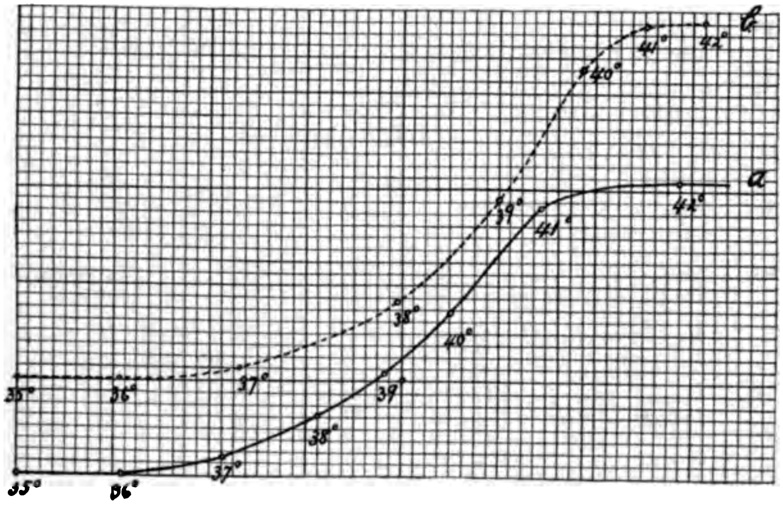

FIGURE 2. One half the original size. Curve of heat rigor in: *a*, the normal muscle; *b*, the fatigued muscle irrigated with dextrose.

made to restore the deficient glycogen by supplying the muscle with dextrose in the proportion normally present in the blood. The experiments for this purpose were made in three ways.

1. The method first employed was to fatigue one gastrocnemius muscle completely by stimulation of the sciatic nerve, and then to irrigate it for two hours with saline solution containing 0.1 per cent of dextrose. Ten experiments of this nature showed a complete restoration of normal rigor in the fatigued muscle in but three cases. A curve plotted from one of these records is given here. Five of the remaining seven experiments showed a partial recovery; in the remaining two the irrigation produced no effect whatever. It is only fair to say, however, that in one of these failures the saline solution used for irrigation was not fresh, and in the other the irrigation is noted as not satisfactory.

TABLE VII.

Irrigation with dextrose after fatigue; one muscle normal.

No.		Rigor begun. degree	Rigor ended. degree	Amount of shortening in mm.	Remarks.
I	Normal	33	42	59	
	Fatigue + Irrigation .	34	42	51	
II	Normal	36	42	47	Shown in plotted curve.
	Fatigue + Irrigation .	36	43	51	
III	Normal	37	42	39	
	Fatigue + Irrigation .	35	44	24	
IV	Normal	37	44	51	
	Fatigue + Irrigation .	35	42	44	
V	Normal	40	44	28	Saline solution stale.
	Fatigue + Irrigation .	35	42	9	
VI	Normal	39	46	43	Irrigation not satisfactory.
	Fatigue + Irrigation .	32	43	10	
VII	Normal	40	45	32	
	Fatigue + Irrigation .	32	41	24	
VIII	Normal	36	42	51	
	Fatigue + Irrigation .	34	42	42	
IX	Normal	34	42	42	
	Fatigue + Irrigation .	34	43	39	
X	Normal	36	43	51	Ringer's solution.
	Fatigue + Irrigation .	35	41	48	

2. Both muscles were fatigued at the same time by placing the two sciatic plexuses upon the same electrode. One of the muscles was then irrigated with saline solution containing 0.1 per cent of dextrose. In some cases the other leg was simply ligated; in others it was removed from the body and kept wrapped in filter paper moistened with saline solution. Seven experiments carried out in this manner showed recovery in the irrigated muscle to a greater or less extent in all cases. In one experiment the recovery was apparently complete. A curve plotted from this record is given here.

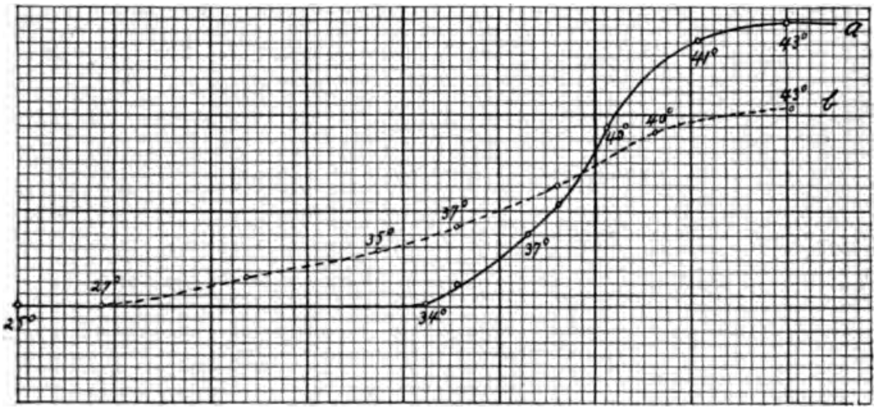

FIGURE 3. One half the original size. Curve of heat rigor in: *b*, the fatigued muscle; *a*, the fatigued muscle irrigated with dextrose.

It will be seen in Table VIII, which gives the results of these experiments, that the period of rest allowed to the fatigued but isolated muscle while the other was undergoing irrigation did not in the least restore the normal rigor.

3. One muscle was fatigued and allowed to go into heat rigor at once, while the other muscle was irrigated with the dextrose while it was being fatigued. Of three such experiments, two showed complete recovery, the third was practically an entire failure.

To supplement these three series an experiment was performed in which after both muscles had been fatigued and one of them irrigated with dextrose they were allowed to go into normal rigor. The fatigued muscle began to contract at once and its rigor was complete in about seven hours; the muscle that had been fatigued and irrigated after fatigue, showed no signs of rigor until the tenth hour, and

TABLE VIII.

Irrigation with dextrose after fatigue; both muscles fatigued.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Fatigue	27	42	38	
	Fatigue + Irrigation .	33	42	42	
II	Fatigue	26	42	35	
	Fatigue + Irrigation .	33	43	47	
III	Fatigue	32	40	31	
	Fatigue + Irrigation .	33	40	45	
IV	Fatigue	31	43	42	
	Fatigue + Irrigation .	36	42	58	
V	Fatigue	30	42	27	
	Fatigue + Irrigation .	30	42	34	
VI	Fatigue	28	44	30	
	Fatigue + Irrigation .	32	43	29	
VII	Fatigue	28	42	39	Shown in plotted curve.
	Fatigue + Irrigation .	36	42	51	

TABLE IX.

Irrigation with dextrose during fatigue; both muscles fatigued.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.
I	Fatigue	32	42	31
	Fatigue + Irrigation .	37	44	50
II	Fatigue	27	40	25
	Fatigue + Irrigation .	39	44	44
III	Fatigue	31	41	42
	Fatigue + Irrigation .	34	44	38

ceased to contract in about twenty-two hours. The amount of contraction in the two muscles was very nearly the same. It should be noted that during this experiment the temperature of the room was unusually high, — 25° to 27° C. This fact probably accounts for the occurrence of rigor in the irrigated muscle as early as the tenth hour, which is sooner than is usual in normal rigor. The same circumstance may perhaps account for the fact that the amount of shortening was no greater in the irrigated muscle than in the other; for both muscles when they were removed from the apparatus at the end of twenty-three hours showed marked evidence of decomposition, and as rigor in the irrigated muscle was not complete until the end of the twenty-second hour, this condition must have been present during its later stages. Unfortunately there was no opportunity of repeating this experiment at a lower temperature.

One other experiment was performed, in which a fatigued muscle was irrigated in the usual manner with a solution of cane sugar instead of dextrose. This irrigation produced no effect whatever upon the ordinary fatigue curve of heat rigor.

It will be seen from the accompanying tables that there are only three cases in which the variation in heat rigor caused by fatigue has been wholly unaffected by irrigation with dextrose. For two of these cases — numbers five and six in table seven — some explanation can be offered ; this leaves only one case of entire failure, — number three in table nine. Thus eighteen out of the twenty-one experiments — more than two-thirds of the whole number — show a restoration of the usual heat rigor, either partial or complete, after irrigation with dextrose. We cannot refer this beneficial action of the dextrose solution to a mere physical change in the liquid, such as increased osmotic pressure, since the experiments with other irrigating fluids make such a conclusion impossible. The sugar probably acts chemically in restoring the normal composition of the muscle substance, and it seems justifiable to infer that the changes in the rigor curve that have been described as the result of fatigue are connected in some way with the using up of the glycogen contents of the muscle.

IV. Conclusions.

1. After prolonged fatigue from electrical stimulation the muscle enters into rigor much earlier, and shortens much less than when not fatigued.

2. This modification of rigor is not due to an increase in lactic acid, or to an accumulation of carbon dioxide, or to the heaping up of other fatigue products, or to the abstraction of calcium salts.

3. It is due to the exhaustion of the glycogen normally present in muscle. For the circulation through the exhausted muscle of a

liquid containing dextrose in the proportion normally present in the blood effects a more or less complete restoration of the normal rigor.

It should be remarked here that it was of course impossible to judge in any experiment when the glycogen contents of the muscle had been restored. To make the experiments comparable it was thought best to use always the same amount (one litre) of the irrigating fluid, and to allow as nearly as possible the same time (two hours) for its passage through the blood-vessels. By this means five complete recoveries in twenty-one experiments have been obtained; and this proportion seems quite as large as could be expected. In the other experiments in which the restoration was only partial, it seems reasonable to conclude that had it been possible to use a more normal circulating liquid, such as frog's serum, in place of the 0.5 per cent saline solution, the restoration would have been more complete.

V. GENERAL OBSERVATIONS.

It has sometimes happened in the course of this investigation that results have been obtained which, while they did not bear upon the main point at issue, were not without interest for other reasons. A brief allusion to these observations seems therefore not out of place.

Nervous system. — The influence of the central nervous system upon rigor mortis has been the subject of repeated investigation. Nagel, Bierfreund, von Gendre, Aust, von Eiselberg, all claim to have demonstrated that the central nervous system exerts a marked influence over rigor. Tamassia, on the other hand, believes himself to have shown positive evidence to the contrary. In the course of the present investigation it was found that in the experiments already mentioned, in which the muscle was irrigated with sodium oxalate, the irritability of the muscle was completely lost, although heat rigor occurred in the usual manner. These results, which are given in Table V, agree with Howell's[1] results obtained on normal rigor. Further, it was observed in these experiments that the irritability which was completely lost to indirect stimulation of the muscle after fatigue, was sometimes restored by irrigation with dextrose; quite as often, however, the irrigation was without effect in this particular. This recovery or non-recovery of irritability was wholly independent of the restoration of heat rigor; for it was observed that recovery in this latter respect might be marked in cases where the irritability of the muscle was very slight; and in two instances where a complete restoration of normal heat rigor occurred in an irrigated muscle its irritability was almost absent. So far then as these results go, they agree, not with the majority of observers, who consider that rigor mortis is under the direct control of the central nervous system, but with Tamassia, who states that it has no influence on the progress, the intensity, and the duration of cadaveric rigidity.[1]

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[1] HOWELL: Journal of physiology, 1894, xvi, p. 482.

Effect of acids and alkalis. — It was mentioned in the early part of this article that irrigation with either lactic or acetic acid caused a delay in the appearance of rigor in the irrigated muscle. On trying the effect of an opposite treatment by washing out a normal muscle with a saline solution containing 0.05 per cent of sodium carbonate, a result exactly the reverse of the former was obtained; rigor appeared in the irrigated muscle at a temperature several degrees lower than in the muscle of the other leg. The extent of shortening was not affected by either acid or alkali. It is well known that the heat coagulation of proteid substances is hastened by the presence of acids and retarded by alkalis; and as the results just mentioned show that these substances affect the condition of heat rigor in a wholly opposite manner, they may be taken as additional evidence in favor of the theory which considers rigor caloris to be a genuine rigor mortis rapidly developed under the influence of the high temperature, and not due to a simple heat coagulation of some of the proteids of the muscle.

Effect of irrigation with dextrose on muscle irritability. — It has been mentioned that in some of these experiments both muscles were fatigued, and one of them irrigated with dextrose while undergoing fatigue. It seemed *a priori* probable that the muscle which was supplied with sugar during its stimulation would require a longer time to become exhausted than the other, which was simply stimulated to the point of fatigue. On the contrary, the muscle irrigated with dextrose became exhausted much sooner than the other; and in one case, where the irrigation was begun fifteen minutes before the stimulation, the irrigated muscle almost immediately refused to respond to stimulation through the nerve. As the stimulation of the muscle in all these cases was effected through the nerve, it may be that the comparatively early loss of irritability in the irrigated muscle was owing to some interruption of the connections between the nerve and muscle fibres — for instance, by injury of the end plates in consequence of the œdematous condition provoked. Whether this was the case was not ascertained by experiments on the direct irritability of the muscle in this condition; so that, under the circumstances, it is not advisable to base any conclusions upon this peculiarity. The tendency of these experiments, however, has been to indicate that there is but little parallelism between the conditions necessary for normal contraction and those required for normal rigor. That the controlling conditions of the two phenomena are not identical is indicated also by other facts which have been referred to briefly in this paper. Thus it has been shown that when a muscle is irrigated with sodium oxalate it loses its irritability to direct as well as indirect stimulation, but its heat rigor comes on at a normal temperature and follows apparently a normal curve. It would appear from this that although calcium salts in precipitable form are necessary to the normal contraction, they play no part in the contraction of rigor mortis. Again, irrigation of a normal muscle with a saline extract of fatigued muscle diminishes or destroys its irritability to direct stimulation, but the rigor curve of such a muscle preserves practically its normal characteristics, — that is, it suffers no diminution in amount and begins at the normal temperature. This fact indicates that although the waste-products of muscle contraction — so-called fatigue substances — either diminish or entirely suppress the normal contraction, they have no perceptible influence on the changes leading to the rigor contraction, the modification of this last phenomenon following on muscle work being due, as this paper is intended to show, to changes in the glycogen-contents of the muscle.

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[1] TAMASSIA: Rivista sperimentale di frenatria e di medicina legale, 1882, viii; quoted in Archives italiennes de biologie, 1882, ii, p. 254.

I wish, in concluding, to express my strong sense of obligation to Professor Howell, under whose direction this investigation has been carried on, for his very kind interest both in the experimental work and in the preparation of this paper for publication.

I am also indebted to Professor Warren of Bryn Mawr College, in whose laboratory the initial experiments were made, and who first suggested to me that I should investigate the influence of fatigue on rigor mortis.

ON THE RELATION OF THE BLOOD TO THE AUTOMATICITY AND SEQUENCE OF THE HEART-BEAT.

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INTRODUCTION.

THE nature of the inner stimulus that arouses the heart contractions is so hidden in obscurity that but few attempts have been made to explain its probable origin, although it may well be supposed that few problems in physiology have offered such inducements to investigation and speculation. To the specialist the problem resolves itself into a study of the metabolism of the heart tissues, and it is needless to say that our knowledge of the chemical structure of the tissues involved and their reactions during metabolism is so great as to restrain effectually all legitimate speculation to very modest limits. Although a final explanation of the cause of the heart-beat seems clearly impossible at present, yet no physiologist perhaps doubts that it will be attained eventually by the persistent use of methods such as are being employed to-day; and every contribution, however incomplete in its results, it may be hoped will add something to the better understanding of the conditions and difficulties of the problem.

For many years indeed the conditions controlling the heart-beat have been studied and analyzed with care by numerous observers, with the result that certain preliminary views with regard to its causation have become more or less accepted in physiology. Some of these ideas, especially in the matter of the relation of the constituents of the blood to the heart-beat, have seemed to the author to be erroneous, and the present paper is written mainly to correct, if possible, certain misconceptions regarding the part taken by the blood, and to point out what appears to be an important, although somewhat neglected factor, in the production of the inner stimulus through which the so-called automatic contractions are initiated. This paper and the accompanying one by Dr. Greene may be considered as a continuation of the work begun by the author some years ago.

It may be accepted without discussion that at each contraction of the heart muscle, as in the case of ordinary striped muscle, there is a chemical change attended by a liberation of energy that formerly existed in potential form. This chemical reaction we can suppose may be initiated by some form of energy falling into the substance of the heart muscle from without, by a nerve impulse, for example; or it may be purely spontaneous or automatic, arising from the intra-molecular movements of some highly unstable substance; or it may follow directly or indirectly from a definite chemical reaction between an energy-containing substance within the heart and some other substance or substances formed periodically within the heart or already existing in the liquids of its tissues. Haller[1] traced the origin of the stimulus to an action of the blood, and in the early part of this century this idea was still prevalent even after the discovery of the existence of nerve cells in the heart. Thus J. Müller[2] speaks of the stimulus as coming from a "constant mutual action which is going on between the blood in the capillaries or between the cardiac nerves and the texture of the heart." Budge[3] supposes that a constant stimulus is in action, coming to the muscle through its motor nerves, but originating in the latter through the action of the blood and not automatically.

Later the source of the inner stimulus seems to have been referred generally to the nerve cells in the heart without specific theories as to its mode of origin. Langendorff,[4] however, makes the specific assumption that the immediate cause of the heart's contractions is to be found in the automaticity of the intrinsic ganglia, and that within these nerve cells the impulse arises as the result of dissociation processes that take place in the heart itself. "Das Lebensproduct der Zelle ist ihr Erreger." According to Langendorff the blood is essential as a nutrient liquid, but possesses no stimulating action. It forms therefore an essential condition for the action of the inner stimulus without itself causing this stimulus directly. Engelmann[5] adopts a similar hypothesis, in that he suggests that there is a continual production of a stimulus within the heart muscle owing probably to the fact that the metabolism of the muscle itself liberates substances which act as stimuli. He further supposes that the continuous stimulus thus provided for, is converted into a periodic stimulus by the fact that these substances are temporarily antagonized or destroyed by other products formed during systole or by a reaction developed during systole. According to this hypothesis it would seem that the substances acting as a stimulus arise mainly from the metabolism of the heart muscle during its period of rest.

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[1] "Qui hos experimentorum nostrorum eventus pensitaverit, is quidem non dubitabit nobiscum pronunciare, causam, quae cor in motum ciet, omnino sanguinem venosum esse. Nam enata ea causa cor movetur, subtracta quiescit, diminuta motus cordis languet, aucta motus intenditur." (See No. 1 in the list of references at the end of the paper.)

Recent authors have agreed quite unanimously that it is useless to attempt to refer the origin of the inner stimulus to the blood or any other agency external to the heart muscle itself or its nerve cells. This conclusion the present author has questioned in a previous paper.[6] Influenced by the important researches of Ringer[7] as well as by results of my own, I have long been convinced that the inorganic salts of the blood and the liquids of the heart tissue, and especially the calcium compounds, stand in a peculiar and fundamental relation to the initiation of the inner stimulus of the heart contractions. The general idea here expressed is not new. In the interesting series of researches from the Leipzig laboratory bearing upon the relation of the inorganic salts of the blood to the heart-beat, this view is implied or stated in several instances, particularly in the paper by Merunowicz.[8] According to this author the automatic contractions of the heart depend upon both the organic and the inorganic constituents of the surrounding liquid. The former furnishes the supply of nourishment from which the energy of the contraction is obtained, while the mineral constituents give to these compounds such a form as enables them to be used within the muscle in the production of a contraction.

Merunowicz and those who immediately followed him were in ignorance of the specific influence of calcium compounds upon the heart's contractility. The discovery of this important fact we owe to Ringer.[7] As the result of his work especially it becomes necessary to consider the inorganic as well as the organic constituents of the blood, and the familiar arguments intended to show that the blood or lymph does not contain substances capable of initiating the stimulus to the heart-beat, seem to the author to fail completely, for, in this sense, no one has shown that a bloodless heart is capable of beating. If, as the author believes, the origin of the inner stimulus is to be traced to the action of certain inorganic constituents in the liquid bathing the heart tissue, it becomes necessary first to prove the efficiency of these constituents in maintaining the heart-beat in solutions from which the organic constituents of the blood are excluded. This is particularly important because of the peculiar views advocated by Kronecker and his pupils in a number of papers.[9] In these researches Kronecker, or those working under his direction, have attempted to demonstrate that the energy of the heart contraction is derived immediately from the serum-albumin of the blood (or lymph) bathing the heart tissue. In the earlier papers of this series the authors were led to erroneous conclusions because of their ignorance of the specific importance of the calcium compounds. For instance, Kronecker[9] believed that he had proved that peptone, which alone or in solution in physiological saline will not maintain contractions of the heart muscle, is rendered capable of performing this function when allowed to remain a short time in the stomach or intestine of a living animal. This result he explained on the assumption that the peptone is thereby converted to serum-albumin, although he furnished no chemical proof that such a transformation occurs. While it is possible that under the given conditions peptone is transformed to some other proteid, and perhaps to something having peculiar nutritive relations to the heart, still it must be borne in mind that under these conditions the solution becomes mixed with the inorganic salts of the gastric or intestinal secretion, peptone, in the case of the gastric juice at least, being an efficient secretogogue. These salts alone, as has been shown,[6] may have been sufficient to give the result observed, and their action should be considered before any assumptions are made as to the conversion of peptone to serum-albumin and a specific effect of the latter on the heart.

So also with regard to the claim made by Martius[10] that a heart irrigated successively with sodium chloride 0.6 per cent and sodium chloride 0.6 per cent made alkaline with sodium carbonate, is brought into a condition in which nothing but a liquid containing serum-albumin can make it beat; for the error of this statement was easily shown after Ringer had called attention to the effect of calcium salts. In the latest paper by White[11] the wonderful efficiency of the calcium salts mixed in certain proportions with potassium and sodium salts is duly recognized, but the contention is still made that the presence of serum-albumin in the liquids of the heart is always an immediate necessity for the production of the heart contractions, these contractions ceasing so soon as the serum-albumin is completely removed. The fact that the ventricle of the frog's heart may continue to beat after steady irrigation for many hours with physiological saline, Martius's liquid and Ringer's mixture of salts, is explained upon the assumption that the heart-beat is maintained during this time by minute residues of blood, with its contained serum-albumin, that have been captured in the capillary crevices of the spongy heart tissue. The point is clearly made in these papers that the heart contains no supply of stored energy in its own substance available for contraction, but derives the energy for contractions directly from the serum-albumin of the blood. In default of the presence of this constituent, contractions of the heart muscle must cease, no matter what other conditions may prevail.

The present author, on the contrary, has been compelled by his experiments to adopt the view of Gaule,[12] namely, that the energy of the heart-beat is derived from material stored in its own substance, material which in the case of the isolated heart supplied with an "inorganic diet" is gradually consumed. For the utilization of this supply of energy, however, certain conditions are necessary, and the principal one of these conditions seems to be the presence in the liquids of the heart of a supply of calcium in some form. In what way the calcium compounds are concerned in the katabolism of the energy-yielding substance in the heart need not be discussed now, nor the antagonistic influence of the potassium compounds. Strong evidence, however, may be presented to show that the calcium compounds have this specific function, and may therefore be regarded in a sense as the source of the so-called inner stimulus.

When Kronecker assumes that a ventricle or a strip of heart muscle, that has been irrigated or bathed for thirty to fifty hours in large quantities of a liquid containing only inorganic salts, continues to beat by virtue of the serum-albumin present in the capillary spaces of the tissue, he makes an assumption that can neither be proved nor disproved positively. By virtue of his hypothesis, traces of serum-albumin far too minute to be detected by the chemical means at our disposal may continue to supply energy for contractions; if, therefore, so long as contractions continue he claims that serum-albumin is present, no one perhaps can definitely prove the contrary. In judging this matter, therefore, we should consider the probabilities in the case, and should adopt the explanation that is the simplest and most direct conclusion from the experimental evidence. It must be remembered in this connection that at no time has any direct evidence been given that serum-albumin is used in or is immediately necessary to the heart contractions. The most that has been done is to show that liquids containing serum-albumin such as lymph or blood (or milk) will best support the contractions of the isolated heart. Considering the complex composition of these liquids, it is somewhat gratuitous to assume without further proof that their favorable action on the heart is owing to the presence of serum-albumin.

It is necessary here to distinguish also between the nutrient action of such liquids and a possible stimulating action. On the assumption that the heart contains a supply of organic material capable of yielding by katabolic changes the energy for contraction, it is perfectly evident that this supply will be consumed in an isolated heart after a shorter or longer interval, and that for further continuance of the power of contraction new material must be formed within the heart by metabolic processes. That the blood and lymph contain substances for the nutrition of the heart and the building up of its energy-yielding compounds, must be admitted on general grounds, as in the case of ordinary striped muscle or other active tissues. It is highly probable, moreover, that this nutritive material consists in part, at least, of proteid compounds; but that this nutritive supply is to be found especially in the serum-albumin of the blood is an entirely unproved hypothesis, although one very commonly made. But granting that this may be the case, that the serum-albumin and possibly the other proteids of the blood furnish the raw material for nutrition, this is quite a different matter from the hypothesis made by Kronecker, that the serum-albumin of the blood or lymph is directly utilized by the heart tissue in contraction, and that contraction is impossible if the surrounding liquid does not contain this substance. One may grant, indeed one must grant, that the blood supplies nourishment to the heart; but in addition it is possible that by virtue of some of its constituents it may be concerned in initiating the heart contractions, — in furnishing, in other words, the conditions for the production of the inner stimulus.

As opposed to Kronecker's hypothesis and in support of the view that the immediate cause of the heart contractions is to be connected with the inorganic compounds in the liquids of the heart, and especially with their calcium constituents, attention may be called to a number of facts that have been described by different investigators. It is not claimed that any or all of these facts give indisputable proof of the view here advocated, but so far as the author can judge, this view gives a reasonable explanation of these facts as a whole.

When the whole heart of a frog or terrapin is isolated and is irrigated freely with a continuous supply of saline solution (0.6 to 0.8

per cent NaCl) it ceases to beat within a comparatively short period, varying perhaps from one half to three or four hours. If this solution is then replaced by Martius's liquid, physiological saline made alkaline with sodium carbonate (3 to 5 mgms. to each 100 c.c. of saline) and the irrigation is continued, further contractions may be obtained, the duration of which is also quite variable, the maximum being perhaps about three hours. If then the Martius's liquid is replaced by a Ringer's mixture of calcium, potassium, and sodium salts, the heart will continue to beat for many hours. The precise time that the contractions will keep up on this last solution seems to depend partly on the composition of the mixture itself and partly on the condition of the heart. That the latter factor must influence largely the duration of the contractions follows as a necessary result from the hypothesis that the isolated heart beats on the supply of material stored within its substance, since this supply will vary with the previous nutritive condition of the animal. The very great difference in duration of contraction shown by different hearts under conditions otherwise the same, is quite what we should expect upon this hypothesis. But in addition, it is easy to show that the duration of contractions upon Ringer's mixture is often greatly influenced by the composition of the solution. The usual mixture that has been employed in this laboratory as imitating most closely the composition of the blood, has consisted of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.03%. After an isolated heart has ceased to beat upon this mixture, it often happens that increasing gradually the percentage of $CaCl_2$ will call forth renewed contractions for hours. Upon these successive solutions containing only inorganic salts, the frog's heart will continue to beat in favorable cases for as much as thirty hours, and in the case of the terrapin's heart for even a longer period. It is to be remembered that during this entire period the heart is irrigated freely with the solutions and that liquid that has passed once through the heart is not used again. During such an experiment, therefore, the heart will be irrigated with several litres of solution. Similar results have been obtained upon small strips of muscle from the ventricle of the terrapin, as is reported by Greene in an accompanying paper, and additional results of the same character on the muscular tissue of the venae cavae will be reported in detail below. Now to explain the long-continued contractions on the Ringer's mixture, under the conditions described, on the hypothesis that serum-albumin or any other organic constituent of the blood is still contained in the blood-spaces of the tissue in sufficient quantities to supply the energy for the contractions, seems to the author very improbable, and demands something more than its mere statement to render it acceptable. The more probable view surely is that the liquid irrigating the heart during this period is itself in some direct connection with the causation of the beats, particularly when it is found that the composition of this liquid must be of a definite character.

Additional facts that tend in the same direction are as follows: Aqueous extracts of the thoroughly dried residue of blood, or of milk, containing the inorganic salts and other aqueous extractives, but only scarcely detectable traces of proteid, may support the contractions of the heart of the terrapin for eleven hours, and more, when the heart has been previously washed thoroughly with physiological saline for an hour or more, until the beats have become very feeble.[6] So, too, Merunowicz[8] reports that extracts of the serum, after precipitation with alcohol, will revive the ventricle after it has been exhausted by physiological saline. More conclusive still, if the serum, after precipitation with alcohol, is evaporated to dryness and incinerated, the aqueous extract of the ash, containing presumably no proteid at all, exercises almost as favorable action as the whole serum when used upon a ventricle previously exhausted with normal saline. On the other hand, blood serum, from which the precipitable calcium has been removed by the addition of a slight excess of sodium oxalate, behaves toward the terrapin's heart much like simple solutions (0.6%) of sodium chloride,[6] in spite of the fact that the proteids and other constituents, except the calcium salts, are unchanged. It may be urged against this last experiment that the small excess of sodium oxalate acts as a poison, preventing the normal effect of the other constituents of the serum. This objection is difficult to meet, since it is practically impossible in such a liquid to precipitate exactly the calcium salts without having an excess of oxalate, or, on the other hand, to exactly remove the excess of oxalate without introducing an excess of calcium. That the small excess of oxalate has a specifically injurious action is, however, rendered improbable by the facts that addition of excess of calcium chloride will promptly revive the heart-beats, and, moreover, normal saline solutions (0.6% NaCl), containing about the same excess of oxalate, bring the heart to a standstill only gradually, and after standstill is nearly or quite obtained, the prompt use of a Ringer's mixture will revive the heart-beats, as after the use of physiological saline alone. These latter statements are true only for hearts that are in good nutritive condition to begin with, as is the case also when normal saline alone is used. Those who make such experiments will find that some hearts, presumably those in a previously bad condition of nutrition, after being washed to a standstill with sodium chloride 0.7 per cent, do not revive upon Ringer's mixture, and with great difficulty, if at all, upon blood serum. Again, the action of physiological saline upon the heart is not improved by the addition of at least one of the proteids of blood, namely, paraglobulin. This has been shown by Stiénon,[13] as well as by myself.[6] Similar experiments with serum-albumin have not as yet been attempted.

Interesting experiments of the same general character, but made upon strips of the ventricular muscle, are reported in the accompanying paper by Greene. Some of the facts there stated may be repeated here, briefly, to still further emphasize the point we are making. If the heart of a terrapin is thoroughly irrigated with large quantities of sodium chloride 0.7 per cent until the contractions entirely cease, or become very feeble, a process which may require one or two hours of constant irrigation, and if, then, a slender strip from the apical portion of the ventricle is suspended in a mixture of calcium, potassium, and sodium salts in proper proportions, it will again beat with force and regularity for many (50 to 70) hours. It will be found, moreover, that such a strip will not contract rhythmically for long periods, if at all, in solutions of sodium chloride alone, of sodium chloride plus potassium chloride, of sodium chloride, potassium chloride, sodium carbonate, sodium hydrogen phosphate, or with any of these salts with the addition of dextrose. In fact, none of the salts present in the blood will provoke sustained contractions of a heart, or heart strip, previously irrigated thoroughly with physiological salt solution, unless some calcium salt is also present.

Kronecker would explain all the foregoing instances of prolonged sustaining action of calcium-containing liquids upon the heart or the heart strips, by the assumption that the ventricular muscle still contained in its spongy meshwork sufficient blood and serum-albumin to supply the energy for the heart contractions. Under the conditions specified, such an assumption is improbable to begin with, and in the case of the ventricular strips, Greene has shown that it leads to a ***reductio ad absurdum***, if the amount of work that the strip performs upon an "inorganic diet" is calculated, and the energy required is referred solely to serum-albumin, or other proteids that might be present in the blood-spaces of the heart.

It will be seen that in explaining the beat of the isolated heart, fed only with solutions containing inorganic salts, Kronecker is obliged to emphasize greatly the importance of the spongy character of the musculature as affording capillary crevices in which remnants of blood may be captured, and from which they are washed out with great difficulty. It has seemed to me, therefore, that his views might be tested still further by using parts of the heart where this characteristic of the muscular tissue is less strikingly developed. For this reason, in part, I have performed a number of experiments upon muscular strips, taken usually from the right superior cava of the terrapin's heart, at a point near the union of the vein and sinus. The vena cava, at the position selected, shows on its inner surface a slight network of muscular trabeculæ. The network is, however, so slight and superficial that it would seem to be impossible to contend that the shallow spaces thus formed could protect remnants of blood from being washed away after prolonged baths in large volumes of liquid.

AUTOMATIC CONTRACTIONS OF STRIPS OF VENA CAVA.

The experiments that were made with the strips of vein were of a simple character so far as the technique was concerned. A ring of the vein was cut out at the place selected, and this ring was then opened at one point so as to give a narrow strip of vein with the muscle fibres running longitudinally. This strip was attached to a counterpoised lever so arranged that the weight upon the strip could be changed at will, although as a matter of experience only two weights were used, one giving a load of 500 mgms., and the other a load of 100 mgms. The strip was enclosed in a wide glass tube that could be filled with liquid of any desired composition, and an arrangement was provided to enable the experimenter to draw off the liquid readily from below at any time without disturbing the attachments of the strip. The end of the lever was armed with a delicate strip of thin paper to serve as a writing-point. In this way records of the contractions were obtained upon the blackened surface of a kymographion. Two kymographions were used in the experiments. One of these was especially constructed to make one revolution in something over eleven hours, and was used chiefly to take the records during the night. The other was an endless roll kymographion of the Hürthle pattern arranged to run at its slowest speed, eight to fifteen millimetres per second. In the experiments this kymographion is designated as the fast drum, and records were taken upon it usually during the day or whenever it was desirable to know the rate of beat. A record of minutes was taken upon both kymographions by means of a specially arranged clock.

The records thus obtained of the contractions of the vein strips were often very striking for the force and regularity of the beats. The relations of these contractions to the composition of the surrounding liquid will be referred to subsequently. At present, as bearing upon Kronecker's hypothesis, I desire to emphasize the length of time such strips may continue to beat in baths composed of solutions of inorganic salts. It may be stated that the inorganic salts used were always purified by several recrystallizations, beginning with salts purchased from the manufacturers as chemically pure. The water used was always distilled from glass. A brief record of those experiments in which the longest duration of contractions was observed will suffice to give the general character of the experiments and the results obtained.

Experiment of March 9 and 10, 1898. Strip of vein from the right superior cava of the terrapin. Strip rinsed in Ringer's mixture and then suspended in large bulk of same and attached to a recording lever. The contractions of the strip were magnified six times by the lever. The load extending the strip was 500 mgms. in the beginning, but later was changed to 100 mgms. The composition of the Ringer's mixture used in the beginning of the experiment was $NaCl$ 0.7%, $CaCl_2$ 0.026%, and KCl 0.04%. The strip was suspended at 3.30 P. M., March 9. Began to beat at once, showing also rhythmic tone waves. After 1¾ hours the beats were distinctly more feeble, and a new mixture was substituted similar to the first, except that the $CaCl_2 = 0.036\%$. The beats, at first somewhat incoördinated, subsequently improved greatly in amplitude and the strip was beating regularly at 6.10 P. M. At this time the strip was transferred for the night to the slow drum. The record upon this drum showed regular and strong beats superposed upon large tone waves for the first five to six hours. Afterwards the tone waves disappeared gradually and the amplitude of the beats also decreased regularly but slowly. At 9.10 A. M., March 10, the solution was changed to one containing 0.046 per cent $CaCl_2$. The strip continued to beat regularly and at first with increasing amplitude; subsequently the size of the beats became smaller, and at 4.30 P. M., they were quite feeble. At 4.30 P. M., March 10, the solution was changed to one containing 0.05 per cent $CaCl_2$. The beats at first increased in amplitude, but subsequently decreased in amplitude, becoming invisible on the record after five to six hours. Subsequently a few large beats at a slow rate appeared, lasting for about one half hour. The strip then remained quiet until the next morning, 9.15 A. M., March 11. The solution surrounding the strip was then replaced by terrapin serum; no beats were obtained, but this result was inconclusive, as the serum was found to have clotted. (Terrapin serum often shows this peculiarity of successive clottings.) The total record, therefore, of regular contractions of the strip of vein bathed in mixtures of calcium, potassium, and sodium chloride extended from 3.30 P. M., March 9, to 11 P. M., March 10, an interval of thirty-one to thirty-two hours. Subsequent experience indicated that a longer record would have been obtained if the percentage of calcium chloride had been still further increased. Records of the contractions were kept during the entire experiment. The rhythm was perfectly regular throughout, but towards the end with slowly changing rate. During the first day, March 9, the rate remained constantly at twenty beats per minute. On the

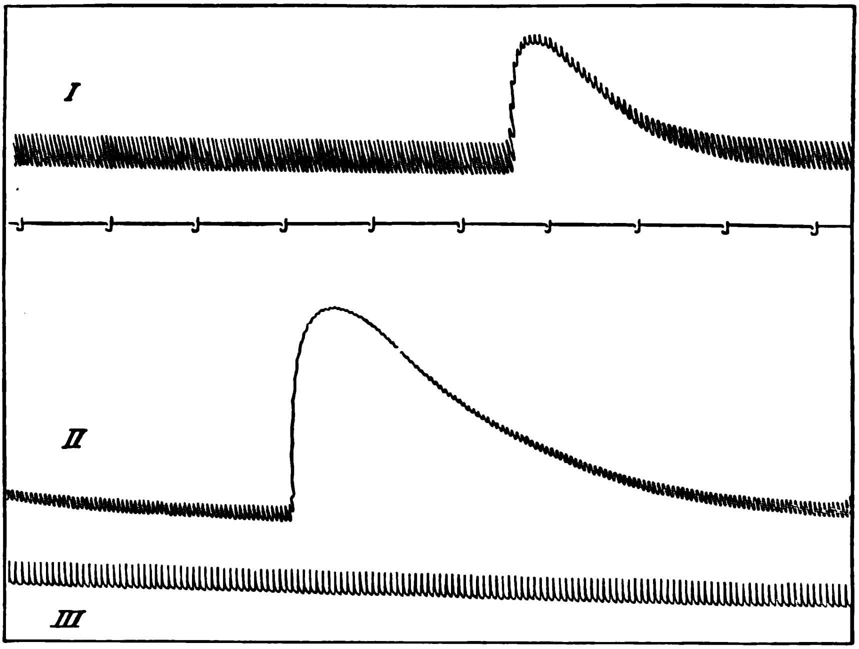

FIGURE 1. Records from experiment of March 9–10, 1898. Seven-ninths the original size. Automatic contractions of a strip of vena cava of the terrapin when immersed in a Ringer's mixture. Curve I, one hour after suspension, $CaCl_2 = 0.026$ per cent. Curve II, three hours after suspension, $CaCl_2 = 0.036$ per cent. Curve III, twenty-three hours after suspension, $CaCl_2 = 0.046$ per cent. The time-record gives minutes, and applies to all the curves.

second day, March 10, the rate steadily decreased, being seventeen beats per minute at 11 A. M., and eight per minute at 4.30 P. M. Specimens of the record obtained at different times are given in the accompanying illustrations.

Experiment March 16 to 19. Strip of vein from right superior vena cava of terrapin. Rinsed in Ringer's mixture and suspended at 11.30 A.M. March 16, in a mixture of NaCl 0.7%, $CaCl_2$ 0.026%, KCl 0.04%; load 100 mgms.

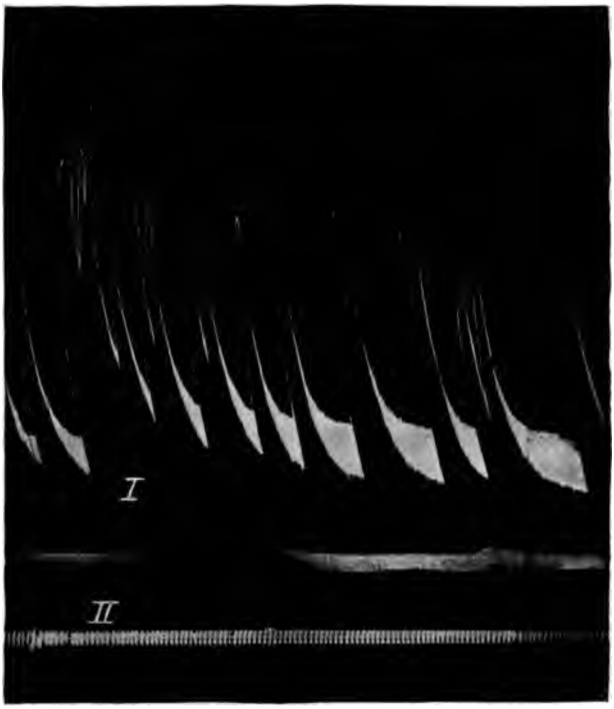

FIGURE 2. Experiment of March 9–10, 1898. Automatic contractions of a strip of vena cava from the terrapin when immersed in a Ringer's mixture. Record on the slow kymographion, giving one revolution in 11 hours. Curve I, 4 to 5 hours after suspension, showing the tone waves. Curve II, 17 to 18 hours after suspension, absence of tone waves. (In part of the record the writing point missed the drum.) The time-record below gives minutes.

Strip began beating at once, showing also large tone waves, rate of beat 14 per minute. At 12.25 A.M. beats feeble; weight increased to 500 mgms., causing an increased amplitude of beat. At 1.20 P.M. rate equal 17 per minute; amplitude of beat 4 mms. At 4 20 P.M. March 16, mixture changed to one containing 0.036% $CaCl_2$, causing increase of amplitude from 1½ mms. to 3½ mms. At 4.40 P.M. load changed from 500 to 100 mgms.; as a result the amplitude of the beat increased to 6 mms., but afterwards gradually decreased to 4 mms. At 6 A.M. March 16 the strip was changed to the slow drum for the night, and the solution was changed to one containing 0.046% $CaCl_2$. The strip beat throughout the night but exhibited curious periods, groups of scarcely visible beats alternating with groups of much larger ones, the beats in the latter groups increasing gradually to a maximum and then slowly decreasing, after the type of the Cheyne-Stokes respiratory rhythm. At 7.30 A.M. March 17, the strip was beating better than during the night, the solution was changed to one containing 0.056% $CaCl_2$. An increase in the amplitude of the beat followed, which soon, however, passed off, the beats again showing periodic groups of larger and smaller amplitude. At 11 A.M. March 17, the solution was changed to one containing 0.066% $CaCl_2$. A remarkable effect followed. The beats increased at once from a scarcely visible size to an amplitude of 3½ mms., showing also an increased rate (not measurable on the slow drum). The strip in this mixture beat well with force and regularity during the afternoon. At 3 P.M. March 17, the strip was transferred to the faster drum, showing a rate of 9 beats per minute. At 4.40 P.M. again transferred to the slow drum for the night. The beats decreased gradually in amplitude during the night, but were still present at 7.15 A.M. March 18. At this time the mixture was changed to one containing 0.076% $CaCl_2$. This solution caused an increase in amplitude, but at 9.30 A.M. March 18, the beats were becoming irregular although of good amplitude. At 10.45 A.M. the solution was changed to one containing 0.086% $CaCl_2$, but with no beneficial effect after an interval of 20 minutes. The solution was then changed to the original mixture containing 0.026% $CaCl_2$. This solution caused a series of irregular beats lasting an hour. The solution was then diluted with twice its volume of NaCl 0.7%, but without effect. At 1.25 P.M. March 18, the bath was changed to one consisting of clear sheep's serum diluted with twice its volume of NaCl 0.7%. The serum caused at once an increase in tone and a return of contractions; finally, regular strong beats at a rate of 16 to 17 per minute and a maximum amplitude of 3½ mms. These beats continued during the night of March 18 with gradually decreasing amplitude until about 9 P.M. After a long period of rest there was a series of small and infrequent contractions between 5 and 7.30 A.M., March 19. These beats stopped suddenly and subsequent exposure to various solutions failed to produce further contractions.

In this experiment it will be noted that the strip of vein beat constantly although with varying amplitude and rate in a bath of inorganic salts from 11.30 A.M., March 16, to 11.45 A.M., March 18, an interval of forty-eight hours. An interesting result observed in this and in other experiments was that as the percentage of calcium chloride was increased, a certain mixture was obtained (in this experiment NaCl 0.7%, $CaCl_2$ 0.066%, KCl 0.04%) in which the character of the beats was suddenly improved for a long period of time, the beats forming a marked contrast to those obtained with solutions having less or more of the $CaCl_2$. Experience has shown that it does not follow that this beneficial result would have been obtained if the same solution had been used at the beginning of the experiment, but it would seem that after a certain period of activity an increase in the percentage of calcium chloride may at a given concentration call forth renewed contractions of remarkable vigor and regularity. This fact serves to emphasize a precaution which has heretofore been overlooked. In testing the effect of a mixture of these salts in sustaining the contractions of heart muscle it is not sufficient to test simply one mixture and when the heart has ceased to beat in this to conclude that it is exhausted so far as any further action of inorganic salts is concerned. In White's[11] experiments, for example, in exhausting the ventricle upon a Ringer's mixture the following proportion of salts was chosen: NaCl 0. 6%, 100 c.c.; $CaCl_2$ 1%, 1 c.c.; KCl 1%, 0.75 c.c.; and $NaHCO_3$ 1%, 1 c.c. When the ventricle had ceased to beat upon this mixture, and in the most favorable cases this happened after nine hours, White drew the unjustifiable conclusion that the supply of serum-albumin in the ventricle was exhausted, and that no further beats could be obtained unless the ventricle was fed with a solution containing serum-albumin. If the author had continued to modify the proportions of salts in his Ringer's mixture he would have discovered doubtless that the ventricle had not been entirely exhausted, or, in terms of the theory he adopts, that some serum-albumin was still present in the heart.

In this experiment it will be noted also that after beating forty-eight hours in a bath of inorganic salts the strip of vein gave good and regular beats for a period of eight hours or more in a bath of diluted blood-serum. A similar result was obtained in other experiments after the strip had contracted for twenty to thirty hours in solutions of Ringer's mixture of salts, and when, apparently, alterations in the proportions of the salts were no longer effective in producing further contractions. Thus in an experiment of March 21–22 the strip was suspended as described above, first, in a mixture containing NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.03%, and this solution was changed at intervals to others with increasing percentages of $CaCl_2$ until the concentration of the $CaCl_2$ was equal to 0.066%. In this case contractions were obtained from 4.45 P.M., March 21, to 3.15 P.M., March 22, an interval of twenty-two hours and a half. At the end of this period the strip was still beating, although irregularly. The strip was then placed in a bath of sheep's serum diluted twice with 0.7 per cent solution of sodium chloride. The result was an improvement in the beats, which was not, however, very marked and lasted only for three hours. In another experiment lasting from April 12 to April 15, the strip of vein was placed first in a mixture of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.04%; and this was replaced subsequently by other mixtures until the percentage of calcium had reached 0.086. The experiment was begun at 6 P.M. April 12. The tone waves for three to four hours were large, but the beats were small and rapid, and about 5.25 P.M. the next day fell into irregular groups after beating regularly for twenty-three and a half hours. The strip was washed for a few minutes in 0.7 per cent solution of sodium chloride and then transferred to a bath of sheep's serum diluted twice with 0.7 per cent sodium chloride solution. The strip soon recovered, giving regular strong beats that increased slowly to a maximum and then gradually grew smaller, becoming too small to record in about ten hours. By changing the serum bath frequently the strip was kept beating feebly for seventeen hours longer, making a total record of about twenty-seven hours, although for some portion of this time during the night the beats were absent or too small to record. Each renewal of the serum during this interval was followed by a temporary increase in the amplitude of the beats.

This favorable action of blood serum, after apparently complete exhaustion upon Ringer's mixture, has been obtained by Greene upon strips of ventricular muscle, and by Mr. Walden, in experiments not yet published, upon the entire heart of the frog. It may well be that in these cases we have an example of a genuine nutritive action of serum. It is possible that the nutrient substances in the serum, whatever these substances may be, are assimilated by the heart tissue and are constructed into new contractile material. The comparatively brief period, however, that the strips continued to beat well in the serum bath is somewhat opposed to this idea. One would suppose that if genuine nutrition had taken place the recovery of contractility would have been of a more permanent character. Another fact that points in the same direction is that after this exposure to serum subsequent treatment with Ringer's mixture of salts failed to provoke contractions. One would suppose that if nutrition had taken place the strip would have reacted to this mixture after the manner of a strip freshly taken from the heart. While, therefore, one cannot deny the possibility of a nutritive action of the serum under the conditions described, it remains possible that this action of the serum may be merely stimulating rather than nutritive. It is possible, indeed it is probable, that in blood serum the inorganic salts, whose importance we are insisting upon, exist in a more favorable condition, either as regards their relative proportions, or the form of their combination, than in the simple Ringer's mixture. This explanation of the temporary recuperative action of serum after previous exhaustion upon Ringer's mixtures is one that may be tested more or less satisfactorily by experiments. At present only a single experiment of this character has been completed, but the results of it may be given here entire, partly because it is indicative of the correctness of the hypothesis suggested, and partly because it falls in line with the other experiments just quoted, in showing the great improbability of Kronecker's view that the contractions of heart muscle are supported only by the serum-albumin present in its substance.

In this experiment the strip of vein was first made to beat in a Ringer's mixture of sodium, potassium, and calcium chlorides until no further contractions could be obtained, the total period being a little more than thirty-three hours. The strip was then transferred to a mixture of similar salts containing also some casein that had been purified by several precipitations. The idea underlying this last modification of the Ringer's mixture was that by bringing the calcium into combination with an organic compound it might be more effective in stimulating heart contractions than when in simple inorganic form.

The casein used in these experiments was prepared from milk by the method first described by Hammarsten, as follows: The milk was diluted with four volumes of water and was then precipitated by careful addition of dilute acetic acid. The precipitate was washed freely, by decantation, in a large bulk of distilled water, several litres of water being used. After draining off the water the precipitate was dissolved in water containing a trace of ammonium hydrate, filtered, and reprecipitated by dilute acetic acid. The second precipitate was again washed thoroughly in several litres of water, and the larger portion of it was then dissolved in a measured amount of one quarter per cent solution of sodium carbonate. The solution thus obtained was used upon the strip of vein, as described below, after being added to a Ringer's mixture of NaCl, $CaCl_2$, and KCl in the proportion of 5 parts of the casein solution to 95 parts of the Ringer's mixture. A portion of the second precipitate of casein was again dissolved in water with a trace of ammonia, filtered, reprecipitated by acetic acid, washed and dissolved in one quarter per cent solution of sodium carbonate. This solution of casein after three precipitations was more dilute than the one used after two precipitations.

As will be seen from the details given below, the strip of vein that had ceased to beat in a series of Ringer's mixtures, again gave regular and strong contractions for a period of nineteen hours in a Ringer's mixture to which the casein solutions had been added, a result as favorable as had been obtained under similar conditions with blood serum. It is perhaps scarcely necessary to add that the good result obtained in this experiment was not due to the sodium carbonate, as was shown by a number of control experiments with Ringer's mixtures containing sodium carbonate. It will also be noted in the experiment given below that after the casein-Ringer mixture had ceased to act upon the strip of vein, diluted sheep's serum was not able to restore automatic contractility, although the strip was still in an irritable condition.

Experiment, April 30 to May 3. A strip of vein was taken from the right superior cava of the terrapin, attached to the lever at 12.55 P.M. April 30, and immersed in a Ringer's mixture of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.04%. The strip began to beat at once, the beats increasing in amplitude to a maximum that was reached in about one-half hour. At 1.30 P.M. the amplitude of the beats was 9 mms. and the rate was 20 to 21 per minute. At 1.45 P.M. the strip began contracting with alternately large and small beats, and tone waves of the usual type appeared. At 3 to 3.30 P.M. April 30 the contractions were again of uniform size, showing an amplitude of 5 mms. and a rate of 18 per minute. At 3.35 P.M. April 30, the record was transferred to the slow drum, and although the contractions were still good and regular a new Ringer's mixture was added, containing 0.036% $CaCl_2$. The contractions continued throughout the night, the amplitude first increasing and then slowly decreasing during eight hours. After this period the contractions were feeble, and fell into groups. At 7.13 A.M. May 1, a new Ringer's mixture was added, containing 0.056% $CaCl_2$. The strip began beating regularly at once, and the record was transferred to the faster drum from 7.20 A.M. to 11.55 A.M. May 1. At 9.50 A.M. and 11.50 A.M. the solution bathing the strip was changed, each time to one containing 0.066% $CaCl_2$. The beats during this period were regular but small, having an amplitude of 2 to 2½ mms. At 11.55 A.M. May 1 the record was again taken upon the slow drum, and at 2.30 P.M. the Ringer's mixture was changed to one containing 0.086% $CaCl_2$. The beats, as the result of this last change, increased in size at first and then became gradually smaller; but regular contractions continued until about 10 to 10.30 P.M. May 1. From this time until seen again at 7 A.M. May 2 the strip showed no beats, but maintained a constant tone. The total time of contracting in Ringer's mixtures with increasing percentages of $CaCl_2$ was therefore from 12.55 P.M. April 30 to 10.30 P.M. May 1, an interval of 33½ hours. At 7 A.M. May 2 the strip was changed from a Ringer's mixture containing 0.086% $CaCl_2$ to a casein-Ringer mixture, containing casein that had been precipitated three times and finally dissolved in one quarter per cent solution of Na_2CO_3. This casein solution had been added to a Ringer's mixture of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.14% in the proportion of 5 parts of the former to 95 parts of the latter. The effect of this solution was to cause a very large increase in tone and regular though small beats. At 9.20 A.M. May 2 the record was changed to the faster drum, a slow increase in tone still continuing. At 9.35 A.M. May 2 the solution bathing the strip was changed to a similar one containing a larger amount, 0.06%, of KCl with the result that while the size of the beat decreased the slow increase in tone continued. At 9.45 A.M. May 2 the strip was changed to a stronger solution of casein (2d precipitation added to a Ringer's mixture containing a larger percentage of $CaCl_2$ — NaCl 0.7%, $CaCl_2$ 0.056%, KCl 0.04%). The beats began to improve at once. At 10.45 A.M. May 2 the bath was changed to a similar solution containing 0.086% $CaCl_2$. The beats at this time had an amplitude of 3+ mms., and a rate of eighteen per minute. The maximum amplitude of beat was reached at 12.45 P.M. May 2, and afterwards the size of the beat gradually decreased. At 5.15 P.M. May 2 the beats were feeble and very slow, two per minute. A new casein-Ringer solution was added at 5.35 P.M. containing 0.126% $CaCl_2$. The beats again increased in amplitude and rate. At 5.50 P.M. May 2 the record was transferred to the slow drum. The beats increased in size to a maximum of 3½ mms. At 9.15 P.M. while the strip was beating well the solution was drawn off, shaken, and again returned to the tube, the change causing a slight temporary increase in the size of the beat. The strip continued to beat but with gradually diminishing amplitude until 2 A.M. May 3. From 2 A.M. to 7 A.M. May 3 the strip gave no contractions. At 7 A.M. changed the casein-Ringer solution to one containing 0.146% $CaCl_2$ and subsequently 0.166% $CaCl_2$, but the strip gave no further contractions. The total period of contractions in the casein-Ringer solution, therefore, was from 7 A.M. May 2 to about 2 A.M. May 3, an interval of 19 hours. After the negative result of the last mentioned casein solution the strip was bathed for a few minutes in 0.7% NaCl solution and subsequently in sheep's serum diluted with twice its volume of 0.7% solution of NaCl. In this solution it was allowed to remain 3½ hours, but gave no contractions at all though showing a slight increase in tone. At 10.45 A.M. May 3 tested the irritability of the strip by mechanical stimulation, stretching it slightly by pressing on the lever; the strip responded at once with a good contraction. The serum was now drawn off and the strip at once began to beat regularly, at a rate of 15 per minute, while suspended in air, at the same time showing gradually a marked increase in tone. The beats became smaller as the tone increased, and finally ceased. At 1 P.M. May 3 the strip was again immersed in the diluted sheep's serum. The result was a marked loss of tone but no contractions whatever during a period of immersion of two hours. At 3 P.M. the serum was again drawn off, leaving the strip suspended in air. Again a series of rapid beats, although this time quite small in size, and a marked increase in tone. The beats in this case soon ceased; the increase in tone, however, continued for an hour and then slowly passed off, the strip returning to its original length in about 10 hours. No beats at all occurred after the few initial ones when the strip was first exposed to air. This strip was weighed while moist at the end of the experiment; its weight was equal to 0.014 grms. Some specimens of the records obtained in this experiment are given in the accompanying illustration (Fig. 3).

It is difficult to apply Kronecker's test of what constitutes a genuinely nutrient liquid to the experiments detailed above. His test as originally stated was that the liquid should be able to call forth and sustain contractions of the heart muscle (ventricle) after the latter had been brought to a complete standstill by successive treatments with normal saline followed by normal saline made alkaline with sodium carbonate. It was stated with great positiveness that any liquid capable of producing rhythmic contractions after this treatment must contain serum-albumin. When, however, White recognized that a ventricle that had been brought to a standstill after treatment by the method of Martius was capable of contracting rhythmically for as much as nine hours longer when supplied with a Ringer's mixture, it became necessary to amend the original statement. Following closely the conditions of his experiment, White was forced to assume that the serum-albumin contained in the capillary spaces of the ventricular muscle was more difficult to remove than had been supposed, and that to remove it and bring the ventricle into a state of complete exhaustion or inability to contract, it was necessary to employ successively the treatment with normal saline, Martius's alkaline saline, and Ringer's mixture in the proportion used by White. After such treatment it was stated with the same positiveness as formerly that no liquid could call forth contractions except one containing serum-albumin. But that this specific conclusion, like the former one, is premature, to say the least, is conclusively shown by my experiments upon the strip of vein, by Greene's experiments on strips of ventricular muscle, and by experiments made in this laboratory by Mr. Walden on the whole heart of the frog. For after exhaustion of the heart muscle by the method employed by White, the simple expedient of increasing gradually the percentage of calcium chloride in the Ringer's mixture may call forth rhythmic contractions for many hours, — the length of time varying with different hearts and depending presumably on the previous condition of nourishment.

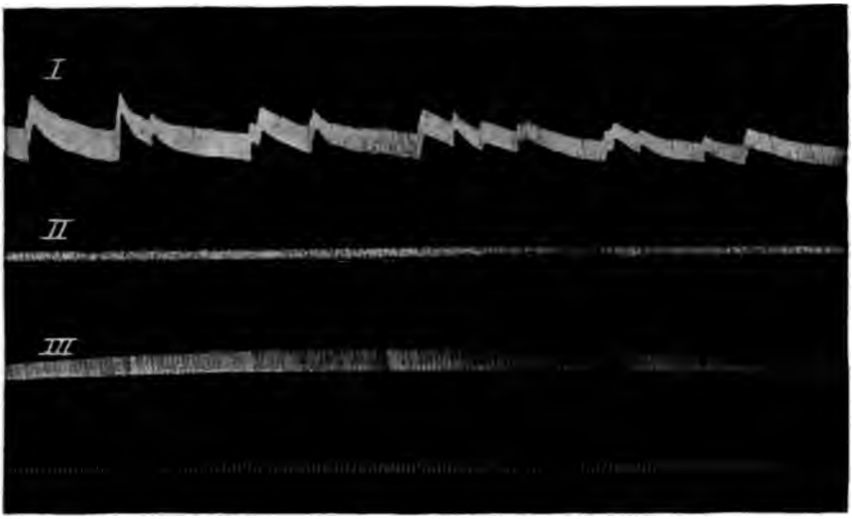

FIGURE 3. Experiment April 30 to May 3, 1898. Seven-eighths the original size. Automatic contractions of a strip of vena cava of the terrapin when immersed in a Ringer's mixture. Records on the slow kymographion giving one revolution in 11 hours. Curve I. Five hours after suspension. $CaCl_2 = 0.036$ per cent. Curve II. Twenty-seven hours after suspension. $CaCl_2 = 0.086$ per cent. Curve III. Fifty-one hours after suspension. The strip at this time was in a bath of Ringer's mixture containing casein (2d precipitate). $CaCl_2 = 0.126$ per cent. The time record on the bottom line gives minutes, and applies to all the curves.

Whatever may be the explanation of the action of these liquids, the fact that a simple strip of vein continued to beat continuously for forty-eight to fifty-two hours, in liquids that were frequently changed and contained no serum-albumin or related proteid, serves to render the hypothesis of Kronecker in the form stated highly improbable. It is scarcely conceivable that the strip of vein soaked for hours in relatively large quantities of liquid should still contain sufficient blood in its substance to supply the energy for such long-continued series of contractions. If one objects that the necessary serum-albumin, although not contained in the blood-spaces of the muscular tissue, may still have been present in the liquids of the tissue, that is, in the tissue lymph, then we are met by a new hypothesis. Apparently it would not be possible to remove completely the serum-albumin from the tissue lymph both extra-cellular and intra-cellular, but if those who believe that serum-albumin furnishes immediately the source of the heart's energy, fall back upon this unprovable hypothesis, one may be justified in insisting on some direct evidence that serum-albumin is directly necessary to, or in any way connected with the contraction of the heart muscle. No positive evidence whatever of this character has yet been submitted. On the other hand, when one considers the wonderful efficiency of Ringer's mixture of inorganic salts in maintaining contractility in the heart muscle, it seems impossible to avoid the conclusion that the energy for these contractions is not furnished by any substance contained in the blood-spaces of the heart, but by material contained within the heart tissue itself. Whether this material is a part of the organized protoplasm of the muscle, or is contained in the liquids of its sarcoplasm or the interstitial lymph, it is not worth the while at present to speculate. It is contained within the heart substance in amounts varying in the hearts of different animals, and, as long as it is present, so-called automatic contractions may be produced if only the proper stimulus is supplied. This position, I take it, is similar to that adopted by Gaule, but disputed by Kronecker, namely, that the heart beats at the expense of its own substance.

As to the nature of the normal stimulus, White[11] admits that the presence of calcium is a necessary condition for contraction. The various experiments described or referred to in this paper upon the effect of artificial mixtures in sustaining the contractions of heart muscle agree in proving the same fact, namely, the necessity of the presence of calcium compounds, and it seems to the author that they indicate, if they do not prove, that the calcium compounds enter into a reaction of some kind with the material furnishing the energy of the contraction, and thereby produce the inner stimulus. As Ringer[7] first showed, the calcium compounds alone, in solutions of sodium chloride isotonic to the blood, are not capable of maintaining a constant rhythm for a great length of time. The ventricle or strip under such conditions shows a tendency to hurried contractions that pass into a state of strong tone. It would appear, therefore, that while the calcium is probably concerned in some way with the reaction that leads to contraction, yet for the production of the rhythmic sequence of full contraction and full relaxation, a combination of potassium and calcium salts in isotonic solutions of sodium chloride is requisite. We have no facts at present, so far as the author is aware, that enable us to explain, even by a provisional hypothesis, the nature of this supposed interaction of the calcium and the potassium compounds.

THE RELATION OF THE NORMAL SEQUENCE OF THE HEART-BEAT TO THE COMPOSITION OF THE CIRCULATING LIQUID.

In the vertebrate heart each beat begins normally in the great veins leading to the heart and thence spreads to auricle and ventricle. The pulsations of the venæ cavæ or pulmonary veins have been described by a number of observers, and in recent years have been studied with care by Tigerstedt and Strömberg[14] and by Engelmann.[5] According to the latter, the three venæ cavæ and the sinus in the amphibian or reptilian heart beat simultaneously or may so beat, the initial contraction of a heart systole beginning spontaneously in any portion of the venous end of the heart. The subsequent contractions of auricle and ventricle, on the contrary, are not spontaneous, but are initiated by an impulse that starts in the great veins and spreads thence to the auricles and ventricles. The fact that the heart systole always under normal conditions begins at the venous end of the heart and spreads in an orderly manner to the arterial end Gaskell[15] has endeavored to explain by showing that the muscle at the venous end possesses greater rhythmic power. He suggests that in the veins and sinus the structure, arrangement, and properties of the muscle cells are similar to what is found in the primitive contractile tube. The muscle cells of this part of the heart therefore retain the primitive rhythmicity of the heart of the young embryo to a greater degree than the more specialized tissue of the auricle and ventricle.

Influenced by the idea advocated in this paper that the inorganic salts of the blood and lymph of the heart play an essential part in initiating and controlling the heart contractions, the suggestion naturally arose to test the reaction of different parts of the heart toward

Ringer's mixtures of varying compositions, toward serum with varying dilutions, and other liquids. It seemed possible that in this way the greater rhythmicity of the venous end of the heart might be brought into causal relations with the normal composition of the circulating blood, and our understanding of the invariable sequence of the heart-beat advanced a step nearer to its ultimate explanation. Upon consulting the literature in connection with the experimental work undertaken for the purpose described, I have been much impressed with several facts discovered by previous workers that have been more or less overlooked, but which seem to me quite significant in their bearing upon the point under discussion. These facts tend to show that the ventricle when fed with undiluted blood or serum is not capable of giving a series of automatic contractions, but if the blood or serum is diluted with normal saline or replaced entirely by saline or other so-called indifferent liquids the ventricle may be made to give long-continued contractions.

Merunowicz[8] (1875) noticed that the apex of the frog's heart, which normally gives no contractions when fed with undiluted serum, may be made to beat if the serum is diluted four times with physiological salt solution. An experiment is described by Merunowicz that illustrates this difference in behavior of the ventricle, and is further interesting in that it is substantially the same experiment as that performed later by Aubert. In this experiment the heart was kept beating for an hour upon the above mixture of serum and physiological salt solution. The apex was then tied off, with the result that it continued to beat without interruption, although in the normal animal with the heart filled with its own blood such an operation is followed by a stoppage of the apex beats.

This difference between the action of undiluted serum and serum diluted with physiological salt solution upon the contractions of the apex of the frog's ventricle is more clearly brought out by the well-known experiments of Aubert.[16] Aubert made use of the clamping device employed by Bernstein. The apex of the ventricle was clamped off for a number (60) of seconds, with the usual result that when the clamp was removed the apex remained motionless although the rhythm of sinus, auricle, and basal portion of the ventricle was undisturbed. If now a little physiological salt solution was introduced into the ventricle through a cannula placed in the inferior vena cava, the apex began to beat in a few minutes with a rhythm independent of that of the rest of the heart. Then while all parts of the heart were beating upon the saline mixture, if undiluted serum was again introduced into the heart the apex soon became quiet, with the exception perhaps of an occasional strong contraction while the remainder of the heart continued its rhythmic pulsations. This alternation of serum and physiological salt solution with the above described effects upon the apex could be repeated a number of times.

The present author has had his attention called very often in experiments made during the last four years to this peculiarity in the reaction of the ventricular muscle. Strips of the apex of the terrapin's ventricle suspended by Gaskell's method in a moist chamber will rarely give spontaneous beats, although soaked with their own blood. With the species of terrapin that I have used spontaneous beats indeed were never obtained, although the strips were stimulated rhythmically for hours in the way recommended by Gaskell for strips from the heart of the tortoise. If, however, such strips are suspended in a bath of physiological salt solution, a series of spontaneous beats will always be obtained unless the nutritive condition of the heart is very poor indeed. The beats produced in this way begin a variable time after the immersion in the salt solution, and exhibit a definite curve, the peculiarities of which are described in detail by Greene in an accompanying paper. In the same paper the author proves that strips of ventricle suspended in the animal's own serum do not usually beat. An occasional strong contraction may occur, but not a rhythmic series. The strip may remain perfectly quiet for hours although quite irritable, and at any time it may be made to give a series of beats by diluting the serum sufficiently with physiological salt solution. It will be noted that this latter result is really the same as that obtained by Aubert[16] in clamping off the apex of the frog's ventricle and changing the serum in the heart cavity to physiological salt solution.

Such results lead us directly to the conclusion that the ventricular muscle of the frog's or terrapin's heart does not contract spontaneously when soaked in its own blood or serum, or to express it in another way, the ventricular muscle under such conditions is not capable of automatic activity. The whole heart, on the contrary, beats when filled with its own blood or serum, or when supplied artificially with the blood or serum of other animals. It follows, therefore, that other parts of the heart toward the venous side are capable of automatic activity when bathed in blood or serum. By means of experiments made on strips from the large veins opening into the heart, I have been able to show this difference in reaction between the tissues at the two ends of the heart in a very striking way.

In these experiments strips of the right superior vena cava, of the apex of the ventricle, and usually of the auricle also, were taken from the same animal and attached in the way previously described to recording levers. Usually the strip of vein was placed under a tension of 100 to 500 mgms., that from the auricle under a tension of one gram while that of the ventricle was loaded with two grams. The strips were immersed in a bath of serum from the blood of the same animal, or in horse's or sheep's serum, or finally in baths of Ringer's mixture of inorganic salts in varying proportions. Careful records of the contractions obtained were taken upon the kymographion during a period of from one to three days. In all, seventeen experiments of this character were completed, but as the records are voluminous on account of the long periods of time required, it is perhaps sufficient to state briefly the character of the results obtained and illustrate these results with specimens of some of the curves.

When immersed in undiluted serum either of the terrapin's or horse's blood, a strip of muscle from the apex of the terrapin's heart does not usually give rhythmic beats. A few isolated strong contractions may occur, but these are rare and take place at long intervals, and in my experiments usually happened immediately or shortly after immersion. As a rule the strip remains perfectly quiet for hours, although very irritable toward mechanical stimulation. From this condition of rest it may be aroused into rhythmic activity by substituting physiological salt solution for the serum, by diluting the serum sufficiently with the physiological saline, or by adding a certain excess of calcium chloride to the serum. Diluting the serum with 0.7 per cent solution of sodium chloride was usually more effective in producing a long series of contractions than the addition of calcium chloride. The amount of dilution required to start the strip into a sustained series of contractions varied in different cases. Sometimes dilution with an equal volume of the 0.7 per cent solution of sodium chloride sufficed, but in other cases dilution with twice the volume or more was necessary. The same variation was observed in the amount of calcium chloride which it was necessary to add to produce contractions; addition of as much as 4 c.c. of a one per cent solution of calcium chloride to 100 c.c. of serum was in some cases entirely ineffective, while 6 or 8 c.c. would call out a series of beats.

A strip of the right superior cava near the sinus on the other hand usually gave rhythmic beats for a variable period, when immersed in undiluted serum. In some cases visible contractions disappeared in two hours or less, in other cases the strip maintained beautifully regular and strong contractions for as long as 24 to 30 hours. A specimen of one of these records is reproduced in Fig. 4. In this case the strip of vein was bathed in serum from its own blood. The illustration shows not only the regular and sustained rhythm of the beats, but also the marked variations in tone, which were practically a constant phenomenon in the records taken with the strips of vein. Whether the strip was immersed in serum or in a Ringer's mixture these marked variations in tone were observed. In some cases they were very pronounced and developed at long and irregular intervals. In other cases they were less extensive or occurred at short intervals and with almost perfect regularity. If the strip was beating, the contractions were superposed on the tone waves, growing smaller as the tone of the strip increased and increasing in amplitude as the strip relaxed. When beats were absent the tone waves were still present, as a rule, and indeed in some cases exhibited an extraordinary range (see Fig. 5).

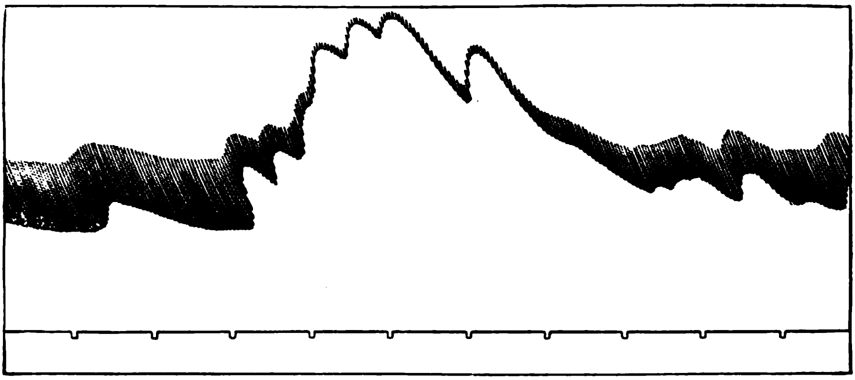

FIGURE 4. Automatic contractions of a strip of vena cava of a terrapin when immersed in a bath of its own serum. Seven tenths original size. Experiment of March 6, 1898. The time record indicates minutes.

In the longer experiments, especially in those in which the bath was composed of Ringer's mixture of salts, it was observed that the wave-like variations in tone increased in amplitude for some hours and then slowly passed off, becoming both smaller and less frequent, so that after twelve or more hours the strip might be beating with as little variation in tone as in the case of the ventricular strip. In this last condition the vein strip was still liable to marked changes in tone if the conditions to which it was exposed were altered. A careful study was not made of the nature of these conditions, but it was observed among other things that aërating the liquid or exposing the strip to air caused usually a strong increase of tone. The susceptibility of the muscular tissue at the venous end of the heart to changes in tone is evidently one of its most striking properties, and one that merits especial study, since it is possible that under normal conditions it may exercise an important regulating influence on the blood flow through the heart. This point, however, was not studied in this investigation, and is mentioned here only incidentally because of the constant appearance of tone waves in all the records.

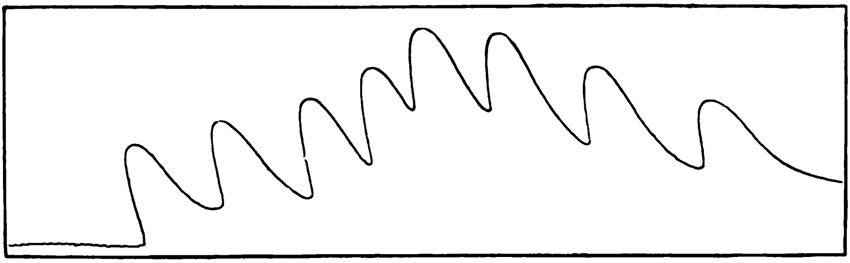

FIGURE 5. Specimen of rhythmic tone waves superposed on a larger tone wave. Seven elevenths the original size. Strip of vena cava of terrapin in a bath of Ringer's mixture consisting of NaCl 0.7%, KCl 0.03%, $CaCl_2$ 0.026%. The strip was giving very small beats just visible in places on the record. Experiment, Feb. 7 to 9, 1898.

The facts given above with regard to the behavior of strips of the vena cava and the ventricle in undiluted serum taken in connection with the results described by Aubert and others, justify us in concluding that the normal composition of blood is such as to prevent automatic activity on the part of the ventricle, at least in the frog and terrapin, while, on the contrary, it induces automatic activity in the tissue at the venous end of the heart. Whether the action in the latter case is exerted immediately upon the muscular tissue or indirectly through the intrinsic ganglia is a secondary matter from our present standpoint.

Strips of auricle were used in these experiments under the same conditions as those to which the strips of vein and of ventricle were exposed. The results, however, were not so uniform, and in the earlier experiments were complicated by the fact that proper precautions were not observed to ensure that none of the sinus musculature was included in the strip taken. It may be said, however, that in general the reactions of the strips of auricle were somewhat intermediate in character between those exhibited by the strips of vein and ventricle.

In some cases the auricular strip gave rhythmic beats in undiluted serum, but these were slower although much stronger than those given by the vein strip. In other cases the auricular strip remained quiet in the undiluted serum, except for changes of tone, but gave a long series of beats when the serum was diluted with physiological salt solution. Dilution with this latter solution, moreover, seemed to cause rhythmic contractions in the auricular strip more easily, that is, with a smaller degree of dilution than in the case of the ventricular strip. In the matter of tone changes also the auricular strip exhibited intermediate properties. Its tone changes were marked, and indeed practically a constant occurrence in the records taken, but they were less pronounced than in the case of the strips of vein.

In addition to the experiments with diluted and undiluted serum, numerous experiments were made in which the bath was composed only of Ringer's mixture of salts in varying proportions. The results of these experiments showed clearly that it is possible to select such a proportion of calcium chloride and potassium chloride in an isotonic or nearly isotonic solution of sodium chloride as will keep the strip of vein beating for hours, but will cause no contractions at all in the ventricular strip. In a mixture, for example, consisting of $NaCl$ 0.7%, $CaCl_2$ 0.026%, and KCl 0.03 to 0.04%, the strip of vein practically always gave rhythmic contractions together with tone waves that might last for hours (see Fig. 6). In the single experiment in which a negative result was obtained with a mixture of the above composition, it is probable that the strip was injured in some way during the process of removal and suspension, since in this case the tone changes were also very slight and infrequent.

In this same mixture of salts the strip of ventricle as a rule gave either no contractions, or if it contracted at all gave only a few beats or small groups of beats separated by long intervals of rest. From this resting condition the ventricular strip might be stimulated to a rhythmic series of beats either by diluting the Ringer's mixture more or less with 0.7 per cent solution of sodium chloride, or by increasing in it the percentage of calcium chloride.

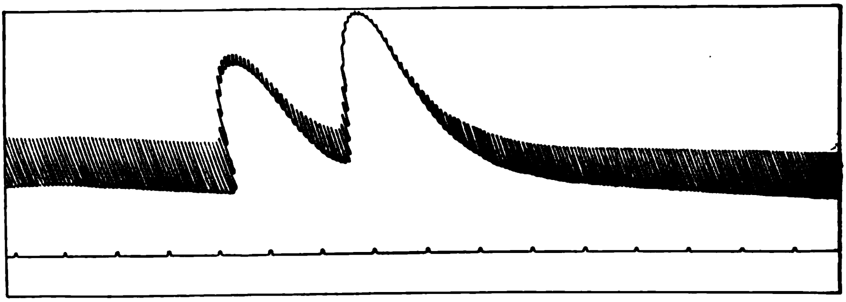

FIGURE 6. Automatic contractions of a strip of vena cava of the terrapin immersed in a bath of Ringer's mixture of the composition NaCl 0.7%, KCl 0.03%, $CaCl_2$ 0.026%. Seven tenths the original size. Experiment of March 21, 1898. The time record indicates minutes.

In addition to the experiments made with strips of ventricular muscle, I have collected a number of observations made during class experiments in which the entire ventricle was employed. In these experiments the ventricle was ligated upon a perfusion cannula, the ligature being laid in the auriculo-ventricular groove. Through the cannula it was irrigated freely with a supply of Ringer's mixture of salts of the composition stated above and under a constant pressure of two to three centimetres. The ventricle was placed in a tonometer filled with 0.7 per cent solution of sodium chloride, and the interior of the tonometer was connected by a wide tube with a test tube suspended by a spiral so arranged that the pressure in the tonometer remained constant in spite of variations in volume of the ventricle. The movements of the test tube were recorded upon a kymographion, and gave the exact changes in volume of the ventricle. The usual result was that when the Ringer's mixture contained 0.026 per cent calcium chloride and 0.03 to 0.04 per cent potassium chloride, the ventricle refused to beat. When, however, the Ringer's mixture was diluted sufficiently with 0.7 per cent solution of sodium chloride, rhythmic, long-continued pulsations ensued. The Ringer's mixture, in other words, behaved precisely like the undiluted serum or blood in the experiments made by Merunowicz and others. The amount of dilution necessary to call forth sustained contractions varied somewhat with the different hearts used.

On the other hand, if the entire heart (sinus, auricles, and ventricle) of a frog or terrapin is isolated and supplied by an artificial circulation of Ringer's mixture of the above composition, the result is that the heart beats quite as well, if not better, than when supplied with serum or blood. We find, therefore, that the reaction of ventricular strips, ventricle, and the entire heart towards a Ringer's mixture of the composition specified is practically identical with the reaction toward undiluted serum. This similarity is most interesting when we remember that the Ringer's mixture chosen contains sodium, potassium, and calcium, in substantially the same proportions as in mammalian blood serum. Whether there is any variation from these proportions in the serum of the terrapin cannot be stated except in the case of the calcium, since complete analyses of the terrapin's serum are lacking. Mr. Greene's determination of the calcium in the serum of the terrapin shows that it exists in the same average percentage as in mammalian serum. For the terrapin's plasma Greene found 0.0131 gms. calcium oxide to each 100 c.c. of plasma, while for mammalian serum Gerlach[19] reports (analyses by Drechsel) for the dog's serum 0.014 gms. calcium oxide, and Abderhalden[20] in some recent analyses gives for ox serum 0.01194 per cent of calcium oxide, and for horse's serum 0.01113%.

It may be assumed that the Ringer's mixture in question contains the bases, sodium, potassium, and calcium in approximately the same percentages as the animal's own blood, and certainly there can be no question that it makes, so far as the entire heart is concerned, a circulating liquid of wonderful efficiency in maintaining rhythmic contractility. The author has in fact been accustomed for some years to use a mixture of practically this composition in class experiments for students in place of dilute serum, and it has always worked very satisfactorily even when the experiments, as in the determination of the effect of temperature on the heart rate, have extended over a number of hours.

From the experiments and results quoted in the first part of this paper the conclusion was drawn that the rhythmic automatic activity of heart muscle is in reality dependent upon the presence of a proper proportion of potassium and calcium compounds in the liquids permeating it, and that of these two compounds the calcium in some way has an intimate connection with the liberation of the stimulus causing contraction. If this conclusion is justified, the facts stated in the second part of the paper serve to make reasonably clear the cause of the normal sequence of the contractions that sweep over the heart from venous to arterial end. As Gaskell and others have shown, the explanation of this regular sequence is to be found in the greater rhythmic power of the tissue at the venous end of the heart, but the cause of the greater rhythmic power in turn is to be sought in the adjustment of the tissue at that point to the relative proportions of calcium and potassium compounds in the liquids of the blood or lymph. This proportion is such that in the heart of the frog and terrapin the blood is capable of liberating a rhythmic stimulus to the muscular tissue of the veins and sinus, but is ineffective toward the ventricular muscle. With such an adjustment the result must be that under normal conditions a heart-systole begins always at the venous end.

On the other hand the ventricular muscle also is capable of so-called automatic activity if only the liquid bathing it have the proper composition, but in blood or lymph or an artificial solution in which the calcium and potassium constituents have the same proportions as in blood it is not automatic. Under these conditions its contractions depend upon a stimulus conducted to it from the auricles, which in turn are stimulated from the sinus, as Gaskell, Engelmann, Krehl and Romberg,[18] and others have contended.

Whether or not these facts hold in the case of the mammalian heart cannot of course be determined without direct experiments on the subject. The well-known experiments of Wooldridge and of Tigerstedt indicate that the ventricle of the mammalian heart, unlike that of the cold-blooded heart, continues to beat, although with a slower rhythm, after separation from the remainder of the heart. This result has been confirmed also by the later experiments of Krehl and Romberg. In all of these experiments, however, a portion of the auricular musculature was left in connection with the ventricle so that the isolation was not complete. Moreover, it is not clear that in these experiments the ventricle continued to beat sufficiently long to establish beyond doubt its power to give automatic contractions under the normal conditions of the circulation. Apparently a much stronger proof of the automaticity of ventricular muscle under normal conditions of circulation is furnished by the interesting experiments of Porter[21] made upon isolated portions of the mammalian ventricle supplied by an artificial circulation of the animal's own serum or blood. In these experiments, however, if I do not misunderstand the author's description, the blood or serum was usually diluted more or less with isotonic solutions of sodium chloride.[1] It is possible of course that the difference in automaticity exhibited by the venous and the arterial end respectively of the cold-blooded heart may be less pronounced although still present in the mammalian heart. On general grounds we should suppose that the cause of the sequence of beat would be the same in both cases.

There is one point mentioned frequently in this paper that merits an attempt at an explanation. I refer to the fact that an entire ventricle or ventricular strip which will not beat when immersed in blood or serum or when exposed to air will give a definite series of beats when bathed in blood that has been diluted sufficiently with physiological salt solution, or better when immersed directly in the salt solution. When the salt solution is used alone the striking feature of this series of beats is the definite curve it presents. The beats begin with a certain amplitude somewhat sub-maximal, increase in size for a short time, and then decrease regularly and rather rapidly to zero. The actual number of beats varies greatly for different hearts, but the series shows always the same characteristic curve. In the accompanying paper by Greene the details of this curve are described and figured. The point that I wish to refer to here especially is the cause of this series of beats. If one accepts the theory of the causation of the heart-beat that I have ventured to propose in this paper, a credible hypothesis may be suggested to explain this interesting effect of isotonic solutions of sodium chloride.

The ventricular muscle is normally saturated with lymph and blood in which the calcium and potassium constituents exist in proportions that neutralize the stimulating effect of the calcium compounds. When, however, the ventricular tissue is placed in a solution of sodium chloride that is isotonic to the blood, a diffusion of the calcium and potassium constituents may be supposed to take place from the liquids of the tissue to the surrounding bath of sodium chloride. If, as may also be assumed, the rapidity of diffusion of the calcium constituents is less than that of the potassium constituents the normal balance between them is disturbed and a preponderance of the calcium compounds results sufficient to stimulate the muscle to contraction. As diffusion proceeds, the contractions will grow smaller and eventually will disappear, when the diffusible calcium and potassium compounds in the tissue fall below a certain amount in consequence of the large bulk of outside liquid against which the diffusion takes place. In this condition of sodium chloride exhaustion, as it has been called, a temporary recovery may be obtained, as Gaule and Martius showed, by the action of small amounts of sodium hydrate or sodium carbonate in isotonic solutions of sodium chloride. The explanation of this partial recovery given by Martius,[10] namely, that the alkali removes more effectually the carbon dioxide of the tissues and thus prevents local asphyxia, is perhaps sufficient. But the recuperating effect of the alkaline saline is slight at the best, and the important fact is that from this condition of exhaustion so-called the muscle may be aroused into rhythmic activity lasting for many hours by exposing it to the action of a liquid containing the proper proportions of calcium and potassium salts, such as blood or lymph or the artificial solution devised by Ringer.

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[1] Dr. Porter has since informed me that he has fed the apex of the dog's heart with dog's blood or serum and obtained rhythmic contractions.

CONCLUSIONS.

1. A strip of vena cava from the heart of the terrapin may be kept in continuous rhythmic pulsation, during forty-eight hours or more, when immersed in baths containing only the inorganic salts, sodium chloride, potassium chloride, and calcium chloride. Since in such preparations there is no mechanical obstacle to the complete removal of blood from the substance of the strip, the result renders improbable the theory advocated by Kronecker that the cardiac tissue beats only as long as serum-albumin is supplied to it by the blood.
2. The muscular tissue of the heart derives the energy for its contractions from material contained within its own substance, and if supplied with an adequate stimulus will continue to contract until this material is consumed, whether or not the organic constituents of the blood are present.
3. Under normal conditions, the stimulus that leads to a heart contraction is dependent upon the presence of calcium compounds in the liquids of the heart; but for rhythmic contractions and relaxations, a certain proportion of potassium compounds is also necessary.
4. An adequate artificial circulating liquid for the whole heart, or for the tissue at its venous end, is a solution of sodium chloride isotonic with the blood, and containing calcium and potassium as chlorides in the proportions, so far as the bases are concerned, found in normal blood. The sodium chloride seems to be essential only in preserving the osmotic relations between the tissues and the surrounding liquid.

5. The muscular tissue of the ventricle of the frog and terrapin is not able to contract automatically when exposed to undiluted blood or serum, or to a Ringer's mixture containing sodium, potassium, and calcium in the proportions present in blood.

6. The tissue at the venous end of the heart (vena cava) is capable of giving sustained automatic contractions in undiluted blood or serum, or in a Ringer's mixture containing sodium, potassium, and calcium in the proportions found in blood.

7. The normal rhythm of the cold-blooded heart depends upon the fact that in blood and lymph the proportion of calcium and potassium compounds is such as to cause rhythmic activity of the muscular tissue at the venous, but not at the arterial end of the heart.

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ON THE RELATION OF THE INORGANIC SALTS OF BLOOD TO THE AUTOMATIC ACTIVITY OF A STRIP OF VENTRICULAR MUSCLE.

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IT is the intention in this paper to investigate more fully the conditions of automatism in the tissue of the heart rather than to discuss the question whether the automatism of the heart resides primarily in the muscular tissue or in the nervous mechanism. Brown-Séquard[1] pointed out more than fifty years ago that the more fundamental question regarding the heart's activity is not which tissue is automatic but why or under what conditions either tissue shows automatism. I emphasize the idea of conditions of automatism with intention, for while one may justify himself in setting forth hypotheses for the explanation of results obtained under the multiple conditions of experimentation, still one must fully recognize the impossibility of reaching a decisive solution of a question which at the present time involves so many necessarily unknown factors.

The influence of the blood on the heart's contraction must necessarily be considered both from the physical and from the chemical point of view. The characteristics of the blood are isotonicity with the living tissue, a certain degree of viscosity, and a complex chemical composition. Since the experiments of Nasse[2] in 1869, on the effects of different strengths of sodium chloride solution on the irritability of the gastrocnemius of the frog, isotonicity with the living tissue has been recognized by all physiologists as a requisite of any artificial solution adapted to sustain the activity of living tissue. In the experiments recorded in this paper, the effort has been made to keep this factor constant by using solutions as nearly isotonic as possible, except in a very few special cases. Certain investigators, notably Heffter,[14] have insisted upon the importance of a degree of viscosity in an artificial circulating fluid. Others have disproved, or at least have thrown grave doubts on the validity of this conclusion;

and until further evidence is brought forth we may safely ignore this property in any investigation on the chemical constitution of the blood in its relation to tissue activity.

As a basis for the preparation of artificial solutions of the salts found in blood chemical analyses of blood or serum as given in the literature of the subject are in many respects incomplete. The most common defect is the lack of information as to the exact forms of the salts present, and the proportions in which they exist as free salts and as salts in combination with organic components. Then, too, such salts as are found only in minute quantities have not been quantitatively determined, although from a physiological point of view they may be of very great importance.

The most abundant salts of blood are those of sodium, potassium, calcium, and magnesium. In experiments up to the present, the action of these salts has been studied mainly upon the heart of the frog. The experiments described in this paper were confined to the terrapin, and were performed exclusively on strips of muscle cut from the apex of the ventricle. The strips were taken either from ventricles filled with blood or from ventricles freed of blood as far as possible by irrigation with normal saline solution. In the latter case the heart was isolated and irrigated through an inflow cannula inserted into the left vena cava and an outflow cannula in one of the aortic branches. The coronary arteries vary much in their origin in the terrapin used, but care was always taken to establish as good a coronary circulation as possible by cutting one of the coronary veins.

From two to four slender strips were prepared from each heart by first cutting off the apical two thirds and then splitting this into the requisite number of pieces. In all, over fifty different hearts were used, giving from two to four strips each, and each strip was submitted to from five to ten distinct changes in the experimental conditions. The effect of each change was observed for periods varying from one to many hours. The average duration of experimentation on a single strip was from forty to fifty hours, although many experiments exceeded seventy-two hours and some reached ninety-six to ninety-eight hours. It will readily be seen that the total time of suspension and the number and character of changes preceding any particular test are most important factors in determining the results of that test, and that the unifying of the results of the entire series of experiments is correspondingly difficult.

METHODS AND APPARATUS.

In 1880 Gaskell demonstrated that a strip of the apex of the terrapin's ventricle would give automatic rhythmic contractions when suspended in a moist chamber and left to itself. This simple experiment offers a method for the study of rhythmic muscular tissue as such. Although an intricate network of nerve fibres interlaces among the muscle cells of the apex of the ventricle, and, according to Berkley,[18] an occasional isolated nerve cell is found in even the apex in the frog and the mouse, still it can scarcely be supposed that a stimulus that acts to produce rhythmic contractions in the isolated strip of the ventricle acts on the terminal nerve elements to the exclusion of the muscle cells. However this may be, whether the automatism resides in the muscle or nerve, the phenomena associated with the rhythm of an isolated strip of muscle seem to admit of a more definite study than the phenomena associated with the activity of the complexly organized heart itself.

In a preliminary experiment made upon a different species of terrapin from that used in the remainder of the experiments, the apex muscle strip of the terrapin's ventricle suspended in a moist chamber remained alive and gave off rhythmic contractions for three days. This preliminary test fully demonstrated the utility of the method, and I have, therefore, adopted this method for the study of the inorganic constituents of blood in their relation to the activity of cardiac muscle. The following pages will, I hope, fully demonstrate the directness, reliability, and simplicity of the method.

My plan of procedure has been, in brief, to suspend a slender strip of the apex of the ventricle (approximately 2.5 cm. long and weighing 0.2 to 0.3 gram) in a moist chamber, attach it to a recording lever under a definite tension, submit it to an artificial fluid by immersing it in the solution or by moistening it in air, and record the resulting phenomena by the lever and by direct observation. The extraordinarily long life of the muscle under such conditions permits a series of experimental changes affecting each strip. By this method one has the very great advantage of placing several strips from the same heart simultaneously in different solutions. The requirements of this method are very simple, and no intricate apparatus is needed. It is necessary only that the muscle be protected from evaporation, and that it be connected with a delicately poised recording lever. The apparatus I adopted has the additional advantage of all the desirable mechanical conveniences in adjustment.

The muscle was suspended in a glass tube fourteen centimetres in length and of a diameter of one and a half to three centimetres, according to the experiment. The tube was closed at the bottom by a rubber stopper containing a small glass cannula, so arranged as to permit the filling or the complete emptying of the tube without disturbing the heart strip. One side of the upper or inner end of the cannula was drawn out and bent into a small hook for the attachment of the lower end of the muscle strip. The upper end of the heart tube was either covered with oiled paper, or — in the tube used for air experiments — was constricted into a small neck with funnel-shaped outer end, which when filled with a drop of water offered no resistance to the movement of the thread passing through it, yet at the same time prevented evaporation from the heart tube. Silk ligatures were tied around each end of the muscle strip, and one thread closely looped over the glass hook at the top of the stopper-cannula while the other was carried to a lever above, or small glass S-shaped hooks were caught into the ends of the muscle strip. The hooks are preferable for the comparatively large ventricular strips.

A light straw lever was used in these experiments. The lever was balanced on a steel rod one millimetre in diameter by means of a glass hub about twenty-five millimetres long, the ends of which were constricted so that the weight of the lever rested on a very small bearing surface. This device reduced the friction of the lever on the steel wire support to a minimum. The fulcrum of the lever was placed between the attachment of the muscle and the writing point, converting the downward pull on the lever into an upward stroke on the recording cylinder. The lever carried a five-gram weight near the supporting glass hub so adjusted that the total weight on the muscle was approximately one gram. The axis of the lever was supported above the heart tube by means of rods and corks so arranged that the lever could be readily adjusted in any plane. The horizontal adjustment was found extremely convenient, since by it the recording lever could be taken from the recording surface or returned at any moment without in the least disturbing other pieces of apparatus attached to the same stand.

A slow drum was found best adapted to the slow rate usually given by the terrapin's ventricular strip, especially when the experiment continued through a series of hours including, perhaps, two or three consecutive nights. The particular drum used for most of these experiments had a circumference of forty-seven centimetres, and made one revolution in eleven hours. When parallel experiments were made, as was always the case except in one or two experiments, two and three heart tubes with levers were attached to the same stand, the levers writing one above the other.

The artificial solutions used in these experiments were in every case made in water distilled in glass. The absolute necessity of this precaution in any series of experiments involving the effects of the inorganic salts of blood on living animal tissue was first demonstrated by Locke,[16] whose work has recently been confirmed by Ringer. These investigators showed that minute traces of certain of the heavy metals, such as copper, dissolved from the copper-receiving tanks so often used for distilled water, may completely obscure the effects of the particular salt experimented with. The water used in my experiments was prepared in part by the method devised by Jones and Mackay,[19] except that potassium bichromate was substituted for permanganate of potassium in the first flask according to a recent suggestion of the authors', and that I used a Jena glass condenser tube instead of the block tin tube used by them. More recently the water used was purified by making ordinary laboratory distilled water slightly alkaline with sodium hydroxide and re-distilling in glass.

The sodium and potassium salts used were purified by repeated recrystallization of "chemically pure" salts. Solutions were made by dissolving a weighed quantity of the dry salt in a measured quantity of distilled water. Stock solutions of one per cent strength were prepared from all salts, except, of course, sodium chloride, and solutions for immediate use were made up from these stocks as needed. In the case of the deliquescent calcium chloride a solution was prepared of approximately the strength required, and the amount of calcium in this solution was quantitatively determined by the usual gravimetric method of precipitation as calcium oxalate and conversion to calcium oxide. From this general solution a one per cent stock was prepared.

In the details of experiments the proportions of the various salts used in any given artificial solution are expressed in terms of percentage.

SERUM.

Before discussing the relation of solutions of the inorganic blood salts to the action of a ventricular strip, let us first consider the behavior of such a strip immersed in its own serum. If an apex strip cut from the heart of a terrapin, *Chrysemys picta*, is suspended in a heart tube and covered with normal terrapin serum, absolutely no rhythmical contractions are developed. If the preparation is not quickly made and is allowed to evaporate slightly during the process, a few single contractions may occur when the strip is first suspended. The number of such contractions does not exceed ten or fifteen, and they occur during the thirty to sixty minutes following suspension.

In five experiments strips were kept in serum from twenty-one to one hundred hours. After thirty to fifty hours an occasional contraction may occur. The longer a strip is kept in serum the more frequent the contractions become, but they do not on an average exceed one an hour in strips kept for a very long time. One strip, number 47*d*, left undisturbed for seventy-six hours in serum, gave during that time eighty-two single contractions, seventy-five of these occurring during the last half of the time. Meanwhile bacteria appeared in the serum; this was therefore drawn off and the strip left suspended in air, though still moist with serum. The muscle at once began to contract with full normal contractions and continued to do so for six hours, when the recording drum stopped and further record was lost. Another strip was kept in serum for ninety-six hours, and gave only forty contractions during about one half of the time, the record being lost at intervals during the experiment. At the end of ninety-six hours this strip was quiet in a relaxed state, but when changed to 0.6 per cent sodium chloride solution it immediately gave a series of rhythmical contractions. These contractions were more irregular in rate than is usual in a sodium chloride series, such as will be described later. They were at first 1.2 to 1.4 cm. high, and gradually decreased to zero within an hour and a half. After one hundred hours a bath of Ringer's solution produced a strong increase in tone and fibrillation, showing that the muscle was still alive and irritable.

From these examples it will be seen that *normal serum preserves the heart strip in good condition for contraction for a very long time, but does not supply the necessary conditions for the development of rhythmic contractions of ventricular muscle.*

It is well known that an isolated apex of the frog's ventricle will not give contractions when the heart is filled with its own blood, a fact made use of since its discovery by Bernstein in 1876 to support the view that the contractions of the heart are nervous in origin. It is significant, therefore, in light of what I shall present later, that the ventricular strip agrees in its reaction to serum with the apex of the frog's heart isolated by Bernstein's method.

Modified serum. — In 1875 Merunowicz[4] found that blood of the sheep or rabbit is most favorable to the development of good contractions in the frog's heart when diluted with 0.6 per cent sodium chloride solution in the ratio of one part of blood to four parts saline. McGuire[5] in 1878 investigated the relative proportions of rabbit serum and 0.6 per cent saline in reference to their sustaining power on the frog's heart. He found that a mixture of serum and saline in the ratio of one to six does not produce maximal contractions, — that one to two is better, while greater concentration of serum is "unfavorable." At the present day, following these and similar results obtained by other observers, it is customary to dilute rabbit or other mammalian blood with sodium chloride solution when it is to be used for artificial circulation experiments, without inquiring why the dilution improves the blood in its adaptability to the production of good rhythmic contractions. I have repeated on the apex strip the experiments of McGuire on the frog's heart, and have other experimental evidence which I hope will show to what dilute serum owes its efficiency. It has already been shown that the terrapin's own serum in its normal concentration and composition as well as mammalian serum is inefficient in developing rhythmic contractions in a heart strip.

Experiment 24, December 11, 1897. A perfectly fresh heart strip was suspended in fresh serum obtained from another terrapin. During the succeeding forty-one hours, twelve contractions were developed. These contractions varied in amplitude from 0.1 cm. to 1.0 cm., and occurred at very irregular intervals. The smaller contractions were submaximal. Sodium chloride 0.6 per cent was then added at successive intervals during ten minutes. After twelve minutes full, strong contractions began which were at first 1.0 cm. high but increased to 1.2 cm. in two or three minutes, then slowly decreased to 0.8 cm. in the two hours following. The rate was quite irregular in this series and the contractions were in groups of varying rates. Ten minutes after the series began one group showed a rate of twenty-eight per minute. As the height decreased the rate became slower and slower. After two hours contractions came only at long intervals and so continued for twenty-four hours.

Experiment number 30 b. An apex strip was immersed in 0.64 per cent sodium chloride solution until the contractions resulting decreased from 1.15 cm. to 0.02 cm. in height. The saline was then drawn off and normal serum introduced, four hours and five minutes after suspension. The strip immediately exhibited incoördinated contractions, and fibrillation. At the same time the strip shortened in consequence of an increase in "tone." In fifteen minutes the fibrillation disappeared and regular contractions of large amplitude began and the tone passed gradually away. The rate of contraction became slower as the contractions became more nearly normal in height, until in fifty minutes the strip remained quiet in a relaxed condition. The muscle was apparently in a state comparable to that of a fresh strip at rest in a serum bath.

After forty minutes of quiet the serum was diluted with 0.6 per cent sodium chloride solution in the ratio of one part serum to two parts sodium chloride. There was no change for sixty minutes, then contractions 1.7 cm. in height began with a very irregular rate. The contractions gradually decreased in rate and ceased in forty minutes. Six hours and twenty-five minutes from the time the serum was first used the serum-saline was further diluted to the proportion of one part serum to six parts sodium chloride. In ten minutes a perfectly regular series of contractions began, and continued for four hours. The rate during this time slowly decreased from 2 to 1.3 per minute, but was otherwise perfectly regular. The amplitude was exceptionally great, 1.7 cm. Between ten hours twenty-five minutes and sixteen hours thirty-five minutes after the serum was first introduced the strip remained for the most part quiet in diastolic state, only occasionally giving a contraction. That the above heart strip was in good condition all the time was shown by the beautiful series procured when later the strip was transferred to 0.6 per cent solution of sodium chloride.

Attention may here be called to the fact that the serum saline series of regular contractions in the above experiment extends over a time as long as or longer than that of many experiments on the frog's heart noted in literature, and that here, as in most of the experiments in this research, the experiment was continued until the after effects were fully determined. For this particular strip I have a continuous record through successive experimental conditions for seventy-three consecutive hours.

From the above examples it may be noted that automatic rhythmic contractions are developed in the ventricular strip by submitting it directly to a bath of serum diluted with a large amount of physiological saline. Also that beats are developed in a strip that has been exposed to solutions of sodium chloride, 0.6 per cent, and then surrounded by serum. That is, it may be assumed that beats occur during that early period in the process of diffusion when the salts of the serum diffusing into the muscle mass may be assumed to be very much diluted. It must also be noted here that a strip that is quiet in normal serum may be made to beat with perfect rhythm and complete and normal amplitude, although for a variable time, simply by increasing the percentage of calcium salts in the serum.

Further, serum will revive activity in a heart strip after it has been thrown into a state of strong tone accompanied by fibrillation in consequence of the action of other solutions, such as sodium chloride solution containing calcium chloride, or a Ringer's solution used after sodium chloride. The exact type of recovery depends upon the degree of general exhaustion of the strip, *i. e.*, upon its total activity since suspension, and upon the length of time that the strip has been in fibrillation. When such a fibrillating strip is changed to serum it immediately begins to increase in activity, giving stronger and more rapid fibrillary contractions. After a longer or shorter period depending on the above mentioned conditions the strip suddenly ceases fibrillation, partially relaxes, and then begins contractions that are normal in type but irregular in rate and not frequent. (See Figs. 2 and 3.) The contractions obtained from strips that are in a more nearly exhausted state are small at first and gradually increase in height during several hours. Almost invariably, however, the contractions revived by serum become more and more infrequent, and practically cease after five to fifteen hours. This condition is again comparable to that of a fresh strip immersed in serum, except that fatigued strips, it must be remembered, have been under experimentation for several hours and have already expended a great amount of energy in muscular contractions.

Finally, a muscular strip beating for a long time in a solution of inorganic salts may ultimately gradually decrease the amplitude of its contractions and pass into a state from which it never recovers its original amplitude. When in this state it is only slightly revived by serum. This is a state of true exhaustion which will be discussed in more detail later. If such an exhausted strip be immersed in pure serum it will give a series of feeble contractions. These feeble contractions are often of quite a rapid rate, but in height do not even approximate the contraction given by the strip before exhaustion. They quickly disappear again, while serum used on a partially exhausted strip revives quite normal contractions that persist a relatively long time. By treating the exhausted strip with the proper combination of inorganic salts a similar slight recovery in rate is produced, but never quite so great as with serum. (See experiment 42.)

Serum will revive a strip from the quiescent state — so-called exhaustion — following exposure to the action of solutions of sodium chloride or sodium chloride plus potassium chloride, such as will be described later; or from a state of tone and fibrillation caused by the use of Ringer's solution containing excess of calcium salts upon a strip exhausted by sodium chloride. In fact, serum will apparently revive a heart strip from almost any unfavorable condition brought about by isotonic solutions of the inorganic salts found in the blood, except possibly that condition of true exhaustion which has been briefly mentioned above and is described more fully in the section on exhaustion.

SODIUM, POTASSIUM, AND CALCIUM SALTS.

Sodium chloride. — Sodium chloride is the most abundant salt in the blood. Its solution in amounts isotonic with the blood has therefore during nearly thirty years been in constant use as the so-called indifferent or normal physiological saline. So far as is now known, it is the only substance that can be used in an artificial circulation medium to preserve the isotonicity with the tissues, and isotonicity seems to be a necessary factor in all experiments on artificial solutions. I have, therefore, in my experiments taken up first the effects of solutions of sodium chloride on the development and maintenance of the automatic contractions of the apex strip of the terrapin's heart.

It was shown long ago by Merunowicz,[4] 1875, that the isolated apex of the frog's ventricle would, after a certain latent period, beat automatically and rhythmically when the heart was filled with 0.6 per cent sodium chloride solution instead of with normal blood or with sheep's blood diluted with 0.6 per cent sodium chloride. Later Aubert,[8] 1881, showed that not only would the isolated apex beat when filled with sodium chloride but that it would again become quiet if the saline was replaced by undiluted blood; and further, that a heart changed back and forth would contract rhythmically or remain quiet according as it was filled with sodium chloride solution or with blood. Aubert's experiments demonstrated at least that normal saline solution is not "indifferent" to living cardiac tissue in the same sense as is serum.

In these experiments I have tried in various ways to show what takes place when an apex strip is immersed in a bath of normal sodium chloride solution. In the earliest experiments 0.6 per cent sodium chloride was used, but later 0.7 per cent was thought to be more nearly isotonic. If a fresh strip saturated with blood be suspended in a bath of 0.6 per cent or 0.7 per cent sodium chloride solution it begins to beat rhythmically after a certain latent period. The length of the latent period, the height of the contractions, and the character of the rhythm and tone vary greatly in different experiments, but the following examples will serve as types.

TABLE I.

Table showing the great diversity of length of the latent period, rate, and maximal height of contractions produced by immersing a fresh ventricular strip of the terrapin in normal solutions of sodium chloride. The height given is the actual shortening of the strip in contraction.

Number of experiment.	Latent period before contractions begin.	Maximal height of contractions.	Rate when the rhythm first becomes regular, together with the maximal rate in some examples.
19 *b*	11 min.	1.3 cm.	12 to 14 per min.
28 *b*	1 hr. 15 "	0.15 "	3 per min.
29 *b*	1 " 30 "	0.2 "	6, increasing to 10 per min.
31 *a*	2 " 35 "	0.82 "	2.2 " " 7 " "
32 *a*	40 "	0.9 "	6 " " 9 " "
39 *c*	25 "	0.7 "	6.5 " " 10 " "
40 *c*	1 " 00 "	0.9 "	4 " " 8 " "
46 *c*	2 " 3 "	0.6 "	4 per min.

The latent period may be only a few minutes, or it may be as many hours. It seems, in general, to be shortened when the bathing solution is renewed often. In some experiments, however, it was found to be long, even though the solution was often renewed. The height of the contractions varies much in different series, although in general it may be said that the longer the latent period the shorter the contractions when they do begin, and the shorter the time they are continued. I cannot at present give any evidence showing any quantitative relation of the rate and height to the latent period. It is to be noted that a sodium-chloride series, when once established, goes through a regular series of changes in the rate and in the height of the contractions. The rate is almost always slow and irregular at the initiation of the series, becomes regular very quickly or after an interval of as much as twenty minutes, then slightly increases in frequency while decreasing in amplitude, until the contractions are reduced to the fraction of a millimetre in height. The height of the contractions in the series is at first submaximal, quickly increases to a maximum, and then very gradually decreases to a millimetre or less, passing into what has been designated as a state of saline exhaustion, the muscle remaining quiet in a relaxed state. If the muscle is left undisturbed in the solution while it is giving the series of contractions, the series decreases in height with beautiful regularity. But if during the series the solution be renewed, a slight increase in the height of the contractions generally occurs, and this is almost always followed by an increase in rate. When the strip has become quiet in sodium chloride solution, renewal of the solution has only a very slight effect, or else no effect at all, in recovering contractions in the strip. If there is any recovery, it is never more than a minute fraction of the original amplitude. A heart strip, saturated with blood, invariably loses tone when suspended in sodium chloride solution. The loss of tone is rapid at first, but becomes less rapid as the experiment

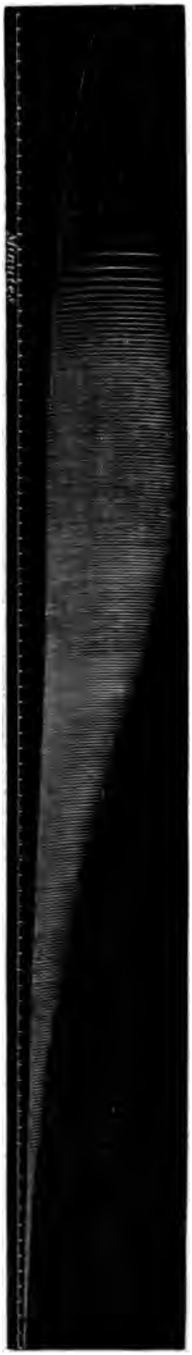

FIGURE 1. — Experiment 35 *a*, Feb. 3, 1898. Curve drawn by a strip of the apex of the ventricle of the terrapin suspended in one per cent sodium chloride solution. About one half the original size. Vertical magnification 4/1. Time in minutes. A latent period of fifteen minutes occurs before contractions commence. The contractions begin at a slow rate, which quickly increases to a maximum of nine to ten per minute, and remains constant during the remainder of the experiment. The height of contractions becomes maximal in fifteen minutes, then rapidly and regularly decreases to less than a millimetre after seventy minutes. It will be noted that the muscle continually loses tone during the experiment, a fact characteristic of all sodium chloride experiments.

progresses. The total loss of tone amounts to one fourth or one third the length of the strip in many experiments. In muscle strips that have previously been treated with solutions of other salts, sodium chloride produces slight or even no loss of tone.

With saline made slightly alkaline with sodium carbonate, I have never obtained a recovery of more than a small fraction of the original amplitude of the beat. This fact is noteworthy in comparison with results obtained on the saline "exhausted" frog's heart by Gaule,[7] Stiénon,[6] Martius,[9] and White.[17] According to my observations, it would seem that the heart-muscle of the terrapin treated with sodium chloride alone passes into a state from which it cannot be revived by alkaline sodium chloride solution. Although in consequence of sodium chloride treatment it gives smaller and smaller beats, and finally remains perfectly quiet in the relaxed state, still, as will be shown, the muscle strip may be thrown into most powerful rhythmic contractions at any moment, if only the proper inorganic salts be added to the saline solution. When the term "exhaustion" is used, therefore, in connection with the saline effect, it must be with the understanding that it applies only to the peculiar condition of quiescence produced by treatment with sodium chloride solution, a condition which might be designated more accurately simply as sodium chloride pause.

The amplitude of the contractions in a sodium chloride series is no criterion of the height of the contractions which the strip will give under the influence of other conditions, *i. e.*, solutions of the other inorganic constituents found in blood. In my experiments, some of the strips which I had condemned as from animals in bad condition, because they gave a poor sodium chloride series, when afterwards subjected to a bath of other inorganic salts gave contractions of unexpected amplitude. Strips cut from hearts which have previously been thoroughly irrigated with sodium chloride solution give only minute beats, or none at all, when suspended in a sodium chloride bath. They react, in fact, like the normal strip suspended in saline at the time when the saline series of contractions is nearly at an end, the exact similarity depending somewhat on the thoroughness of the irrigation. These strips, also, are not truly exhausted, as an experiment to be described later (No. 42) abundantly proves.

The great diversity of reaction to saline solutions exhibited by ventricular strips filled with blood is given at some length, because these are the variations in reactions often ascribed to the individual peculiarities of the animal experimented upon. As a matter of fact, these reactions are only what might have been expected when we consider the cardiac rhythm in its relation to the other inorganic salts of blood. I hope later to suggest a possible explanation of these elements of difference.

If the strength of the sodium chloride to which a fresh muscle strip is subjected is varied, certain interesting phenomena are noticed. In two test experiments of three strips each, the strips in slightly hypertonic solution of sodium chloride began rhythmic contractions after a very short latent period, — a latent period shorter than was ever obtained from the fresh strips in what was assumed to be isotonic solutions, *i. e.*, 0.7 per cent. The strips in hypotonic solutions, on the other hand, were not constant in their behavior. One had an exceptionally long latent period; the other a shorter latent period than that of the control strip in isotonic solution.

TABLE II.

Variations of the strengths of the salts in relation to the latent period and to the rate and maximal height of the succeeding contractions.

Number of experiment.	Per cent of NaCl.	Latent period.	Height of contractions was reduced to 0.1 cm. in	Maximal height of contractions.	Rate when the contractions first become regular. Also the maximal rate.
35 *b*	0.7	38 min.	50 min.	0.8 cm.	8 per min. — constant.
36 *b*	0.7	24 "	70 "	0.6 "	7, increasing to 10 per min.
35 *c*	1.0	15 "	70 "	1.15 "	9.5 per min.
36 *c*	1.0	3 "	42 "	1.0 "	9, increasing to 11 per min.
35 *a*	0.4	3 "	42 "	0.7 "	5 " " 8 " "
36 *a*	0.4	44 "	60 "	0.5 "	6 " " 9 " "

The general cycle of events described above is characteristic of the saline curve of the normal strip. But if the strip is first treated with some other solution or kept in terrapin serum or blood for two or three days, or has been revived from a previous saline treatment and then again subjected to saline solution, its reactions present certain characteristic differences. Figures 2 and 3 illustrate these.

Figure 2 gives the reactions of two strips from the apex of the ventricle of the terrapin, the two from the same heart. Trace *a* begins at *x* with the immersion of the strip in serum twenty-one hours after suspension and following an unfavorable Ringer's solution.

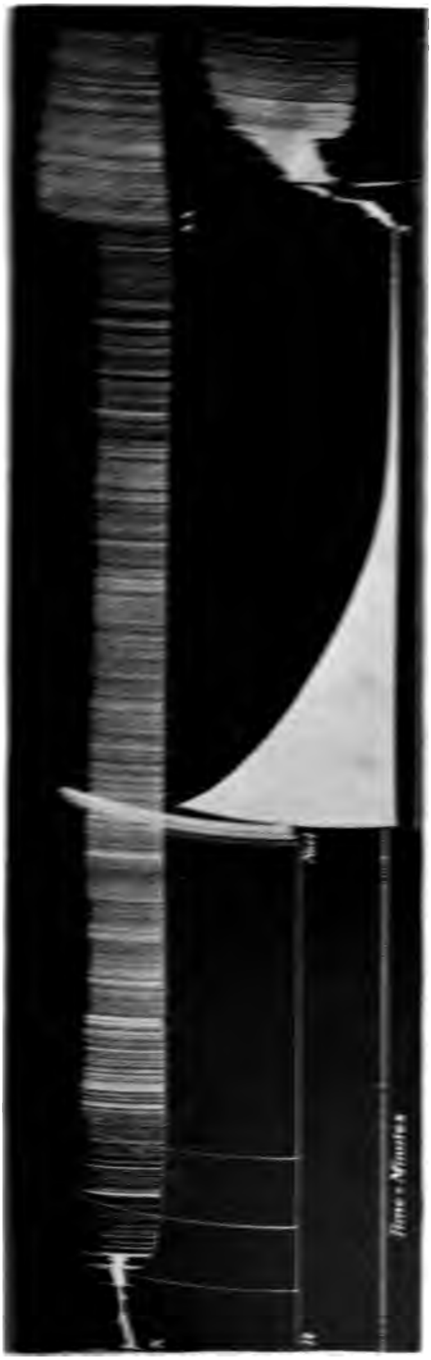

Figure 2. Experiment [illegible] January [illegible] Vertical magnification [illegible] Time in minutes. [illegible] of the ventricle of [illegible] Ringer's solution [illegible] dilute serum. The [illegible] about one half the original [illegible]

The serum immedi- [illegible] after [illegible] minute the [illegible] rate. The [illegible] for seven [illegible] the [illegible] was drawn off [illegible] Ringer's solution [illegible] $CaCl_2$ [illegible] KCl [illegible] [illegible] This was [illegible] by a strong [illegible] the am- [illegible] contrac- [illegible] and a slight [illegible] of rate. [illegible] was recorded [illegible] a suspension of [illegible] hours. [illegible] strip was quiet [illegible] in dilute [illegible] it was [illegible] Ringer's [illegible] NaCl [illegible] KCl [illegible] and $NaHCO_3$ [illegible] No change [illegible] by the [illegible] for three [illegible] and ten min- [illegible] The three iso- [illegible] contractions in- [illegible] the state of the muscle. A change to 0.6 per cent NaCl was immediately followed by a rapid rhythm, eight and more per minute. After four hours in sodium chloride solution the contractions were reduced to a small fraction of the original amplitude. That the activity of the strip was only suspended is shown by the effect of the dilute serum which was used at *S*. Serum produced strong increase in tone with fibrillation, which passed off gradually into well-coördinated contractions. Figure 3 gives the change in the experimental conditions following the effects shown in Fig. 2. In this experiment after thirty hours' suspension the strips *a* and *b* from terrapin No. 30 were immersed in 0.64 per cent sodium chloride solution; *a* had been previously immersed in Ringer's solution, *b* in serum diluted with 0.6 per cent sodium chloride. Strip *a* contracted at the rate of eleven to twelve and *b* at the rate of seven per minute. The similarity between these two traces and the corresponding one in Fig. 2 is very striking and suggestive. After two hours forty minutes in sodium chloride solution, serum produced in *a* an increase in tone with fibrillation, followed by good contractions. Ringer's solution produced in *b* a marked increase in tone with incoördinated contractions and fibrillations, from which the muscle did not recover until serum was again used.

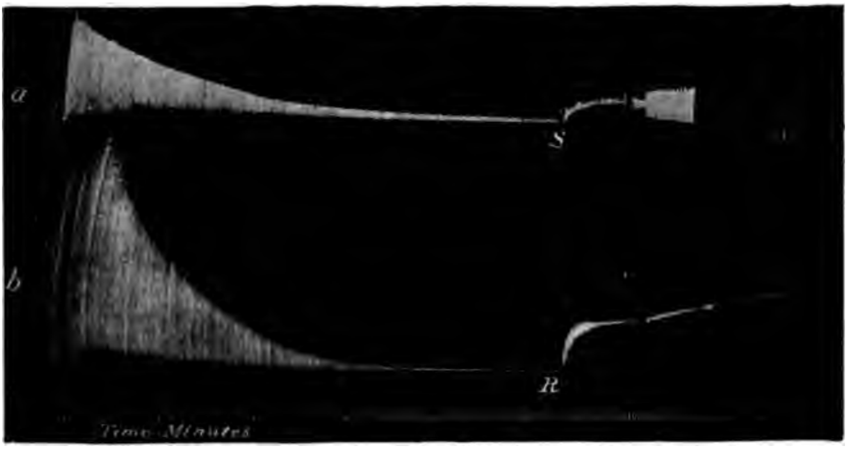

FIGURE 3. Experiment 30, *a* and *b*, January 18, 1898; vertical magnification 2/1; after thirty hours' suspension. Curves drawn by strips from terrapin ventricle immersed in 0.64 per cent sodium chloride solution. Strip *a* had been previously immersed in Ringer's solution; strip *b* in serum diluted with 0.6 per cent sodium chloride. *S*, immersion in serum; *R*, immersion in Ringer's solution. One half the original size.

First and perhaps most important of the characteristic differences illustrated by Figs. 2 and 3 is the shortening or entire absence of the latent period. If the strip has already been submitted to several changes and is still in condition to contract, then saline solution calls forth immediate contractions. Under these conditions also the contractions begin at a submaximal height, usually that of the contractions in the preceding solution, quickly increase to a maximum, and then regularly and uniformly decrease to complete disappearance, the muscle remaining inactive in a relaxed state. In these cases the maximal sodium chloride contractions are maximal for the muscle under *any* condition, a fact in sharp contrast to the submaximal contractions so often obtained from a fresh strip treated with sodium chloride solution. The rate is also much increased, in many experiments reaching twenty or more per minute, a rate too rapid to be distinguished in the records made on the slowly moving drum. The effects present the appearance of a very much heightened irritability, and the longer the muscle has been suspended the more striking is the result.

The rate in sodium chloride remains more nearly constant than in any other solution except, perhaps, very much diluted blood or serum. The decrease in the height of the contractions brought out in a heart strip by a second sodium chloride treatment is almost always perfectly regular and symmetrical. Such regularity in the response of the muscle to a definite salt in solution argues for a definite and regular series of changes in the muscle, either of a physical, *i. e.* osmotic, or of a chemical nature. I shall only briefly call attention here to certain facts which are brought out by this particular group of experiments.

It must be borne in mind that the muscle strip cut from a fresh unwashed ventricle is full of blood, that is, its muscle fibres are bathed in a liquid containing the numerous constituents of blood. When this strip is suspended in a solution of sodium chloride as nearly isotonic with blood as may be, it is to be presumed that diffusion of the salts other than sodium chloride immediately begins. Presumably osmotic currents of water and the diffusion of sodium chloride are reduced to a minimum, but all other diffusible constituents of the blood will tend to pass into the surrounding liquid. The more frequently this surrounding liquid is renewed the more rapidly the process is carried on. When contractions begin, as they do sooner or later, the rhythmical change in pressure greatly aids the process. During the first few contractions the blood is squeezed out of the interstices of the strip in quantities sufficient to discolor the saline solution. When the whole heart is irrigated by saline solution before the strips of ventricle are cut the same process must necessarily go on during irrigation. In this case it is much more rapid, since the saline solution flows through the coronary system of blood vessels. The heart always contracts when irrigated, hence this mechanical aid to the washing out process is most effective in irrigating the whole heart.

Recalling, then, in the first place, that the apex ventricular strip cut from a heart previously irrigated and washed with saline solution will not contract when suspended in a saline solution, and secondly, that a heart strip filled with blood gives a rhythmic series of beats, diminishing rapidly to zero when suspended in a saline solution, and thirdly, that a strip revived by other solutions and again surrounded by sodium chloride solution repeats essentially the same process, my results may be briefly summarized as follows:

1. Sodium chloride in solution in distilled water will sustain the ventricular muscle of the terrapin in rhythmic contractions for a brief time only, one to two hours.
2. The ventricular muscle is not exhausted by sodium chloride solution, although the decreasing amplitude of the series of contractions simulates exhaustion.
3. Sodium chloride solutions stimulate a heart strip to a brief series of contractions of increasing rhythm and decreasing amplitude.
4. A sodium chloride bath does not always develop maximal contractions in a heart strip.
5. Sodium chloride solution produces marked loss of tone in a fresh ventricular strip.

Calcium chloride.—Calcium exists in the blood presumably in combination and as free salts or the corresponding ions. Quantitative analyses of the calcium present in the blood of the terrapin were made in several instances. A large terrapin was bled for such an analysis, and the blood kept on ice a day or more until the corpuscles had settled. From fifty to sixty cubic centimetres of clear amber-colored plasma were obtained from a single terrapin. The plasma to be analyzed was siphoned off into a standardized graduated burette, measured amounts drawn into large centrifugalizing tubes, ammonia added to slight excess, and the calcium precipitated with ammonium oxalate. The precipitate was now left to settle, or else was thrown down by means of a centrifugal machine, washed in distilled water, redissolved in weak hydrochloric acid, reprecipitated, washed until free of chlorine, dried, and finally heated in a platinum crucible until the weight remained constant. Duplicate analyses were made and the results are expressed as grams of calcium oxide in 100 c.c. of plasma. Each tube in Experiment I contained 24 c.c., in Experiment II, 25 c.c. of plasma.

TABLE III.

Determination of calcium in the plasma of the slider terrapin expressed as calcium oxide in 100 c.c. of plasma.

Experiment I. December 4, 1897. $a = 0.0126$ gram, $b = 0.0126$ gram.

Experiment II. December 11, 1887. $a = 0.0133$ gram, $b = 0.0141$ gram.

Mean of four determinations, 0.0131 gram.

This determination is in fair agreement with the results of Gerlach,[20] secured by the same method from dog's serum, namely, 0.014 and 0.0145 gram CaO in 100 c.c. serum, and also with determinations of the calcium in sheep serum made by Dr. Howell in this laboratory by a volumetric method with potassium permanganate, which gave as the mean of two analyses 0.0124 CaO in 100 c.c. of serum.

The mean of the determinations in Table III expressed as calcium chloride is 0.026 gram per 100 c.c. of plasma. This amount was considered as the amount normal to the blood. Chloride salts were used in all the artificial inorganic salt solutions in order to reduce the effect of the acid radical to a constant. One would expect the amount of calcium to vary in different individuals. It is possible, too, that some of the combined calcium may separate off, and if so the above amount would be too large. It will, however, serve as the most available constant in the study of the effects of the calcium in the blood in its relation to the development and maintenance of contractions in the isolated heart strip. In 1883 Ringer[10] first demonstrated the great importance of this element in the activity of the frog's heart. Since that time numerous experimenters have extended and enlarged our knowledge of the physiological importance of calcium in the animal organism. At the present time it is generally recognized that calcium in some form plays an essential part not only in the activity of muscle but also in the clotting of blood, the coagulation of milk, etc.

Ringer demonstrated that calcium does not act alone in maintaining the rhythm of the frog's heart, but that it must be antagonized by potassium salts. I will here first discuss briefly the effects of calcium and potassium salts as such, and later take up their relation to other salts. Isotonic strengths of calcium are imperfectly borne by the cardiac muscle, hence calcium effects must be studied in combination with some other isotonic solution. Of these I have used isotonic solutions of sodium chloride, dextrose, and urea. The last seems injurious, and no contractions have been observed in it. My most reliable calcium effects are, therefore, those obtained in combination with saline effects.

Calcium chloride alone. — Calcium chloride in distilled water in approximately isotonic solution when applied directly to a heart strip throws the muscle into strong tone. No rhythmical contractions are given off for five minutes, — the longest time a strip has been submitted to this excessively strong solution. When the excess of calcium is removed by washing the strip with 0.7 per cent sodium chloride solution, a series of very rapid contractions immediately starts up. The rhythmic contractions are superposed upon a state of strong tonic shortening. No permanent injurious effect follows the use of the strong calcium solutions. If, on the other hand, calcium chloride of the strength found in the blood is applied to a heart strip that is quiet but in good condition for contracting, its application is followed immediately by a rapid and regular series of contractions. The contractions decrease rapidly in height for five minutes, — the longest time a strip has been submitted to this hypotonic solution. In these contractions the relaxation phase is much shortened and the lever does not return to the base line.

The experiments with solutions of calcium chloride alone are not very satisfactory although suggestive. Isotonic solutions on the one hand contain a deleterious amount of calcium, and on the other hand normal strengths of calcium are so strongly hypotonic to the muscle that this physical factor is doubtless a predominant one in determining the results.

Potassium chloride. — Potassium chloride, like calcium chloride, is deleterious when applied to the muscle of the heart in isotonic solutions. Its effects are also complicated by physical phenomena when it is applied in solutions of a strength found normally in the blood. When one per cent potassium chloride solution was applied to a heart strip which was previously contracting rhythmically in dilute serum the heart strip quickly gave one or two spasmodic contractions and then remained quiet in a condition of tone. Afterward, when the excess of potassium was removed by washing the strip with 0.7 per cent sodium chloride, no contractions were developed but the tone spasm passed off. Dilute serum again established a rhythm after a short latent period, and the rhythm appeared perfectly normal in character. From this it will be seen that excessive doses of potassium as well as of calcium salts do not produce a permanent poisonous effect on the ventricular strip when applied for short periods.

In more dilute solutions, *i.e.*, in solutions approximately normal to the blood (0.03 to 0.04 per cent), as determined by physiological reaction and by analyses reported for the blood of other animals, potassium chloride applied to a contracting strip produced quiescence after a few contractions which decreased rapidly in amplitude to complete disappearance. Tonic shortening also followed the use of this hypotonic solution.

SODIUM, POTASSIUM, AND CALCIUM SALTS IN COMBINATION.

Calcium chloride in isotonic solutions of sodium chloride. — If calcium chloride, in an amount normal or subnormal to the blood, be added to isotonic solutions of sodium chloride, certain important results are obtained. This mixture of salts, applied to a fresh, unwashed heart strip, produces a series of contractions after a very short latent period. The series resembles, in general features, the series of contractions given by a control strip in saline alone. But the rate is more rapid in the strip in sodium and calcium chloride solution, and the contractions are maximal from the first, while in the pure sodium chloride solution contractions are usually not maximal for some minutes after the series is inaugurated. The most characteristic effect of the calcium when added to sodium chloride solution is the prevention of perfect relaxation after each contraction. There is a strong rise of the base line instead of the gradual fall so characteristic of the sodium chloride series. If the amount of calcium chloride used on a fresh strip is large, say 0.04 per cent, the rise occurs very soon after the strip begins automatic contractions. The rise begins immediately on the application of the solution when applied to a strip that is quiet after a previous sodium chloride treatment. When a strip ceases to beat in a sodium and calcium chloride mixture it ceases in a state of tone. If the amount of calcium is small, 0.01 per cent, then the increase in muscular tone is only slight. Calcium chloride in solutions of sodium chloride never more than slightly revives a heart strip after it has ceased to contract in a solution of sodium chloride alone.

Calcium chloride in isotonic solutions of dextrose. — Calcium chloride in amount normal to the blood, 0.026 per cent, when applied in isotonic solutions of dextrose to a heart strip previously beating in serum, or in serum diluted with saline, immediately calls forth a series of rapid contractions, together with a strong increase in tone. When the dextrose alone is applied to the heart strip, it calls forth a similar, though less rapid, series of contractions. It is questionable, therefore, just how much of the above effect is due to dextrose and how much to calcium, a point which requires further investigation.

Calcium chloride in isotonic solutions of urea. — Calcium chloride in isotonic solutions of urea produced no contractions in the muscle strip. Urea itself seems injurious to cardiac muscle when applied in isotonic strength, for even serum produces only slight or no recovery after its use.

Potassium chloride in isotonic solutions of sodium chloride. — When a fresh muscle strip is immersed in a solution of 0.03 per cent potassium chloride in isotonic solutions of sodium chloride, either no contractions at all are developed, or, if developed, the contractions are extremely minute, and make their appearance only after an extremely long latent period. The contractions recorded in the exceptional cases occur very irregularly, and are only a small fraction of the height (one ninth in one experiment) of the contractions given after a change to a solution of different composition. These results are in sharp contrast with the behavior of a strip surrounded by sodium chloride alone, or by sodium and calcium chloride solution. Strips in solutions of sodium and potassium chloride almost always show an excessive loss of tone, a result just the opposite to that of strips in solutions of sodium and calcium chloride. Solutions of sodium and potassium chloride have no effect in reviving activity in a strip that has ceased to beat in sodium chloride alone.

Ringer[10] and his students have taken a prominent part in investigations concerning the action of potassium salts on the animal body and on the heart. They have shown that potassium salts applied to the frog's heart produce a slowing of the rate, much dilatation, and ultimate cessation of the rhythm. Ringer was also the first to show the necessity of this salt in antagonizing the excessive stimulating effect of calcium salts. We will now turn to this phase of the subject as applied to the terrapin heart strip.

Sodium, calcium, and potassium chlorides in isotonic solution. — The wonderful sustaining power of sodium, potassium, and calcium salts in solution was first pointed out by Ringer[10] in 1883. In the proportions used in his original formula the solution was shown to revive the frog's heart, and to sustain it in rhythmic contraction for several hours. Ringer afterward substituted the tribasic phosphate for the calcium chloride; but, in so far as the cardiac muscular strip is concerned, I have obtained quite satisfactory results with the chloride.

In my experiments I have striven to secure a proportion among the above inorganic salts that would give the effects on an isolated cardiac strip most nearly approaching that of blood. The facts already pointed out by Ringer and his students, and by Howell and Cooke,[16] seem to indicate that if one could exactly imitate the composition of the blood as regards the inorganic constituents, it would be possible to secure, approximately, the same effects from blood and from the artificial preparation of inorganic salts in so far as the isolated strip is concerned.

By keeping the amount of calcium in isotonic sodium chloride solution constant and equal to the mean of the two analyses of the terrapin plasma, and by varying the amount of potassium, a solution was soon determined which gave fairly constant results. With this solution results were obtained that closely approximated the effects of a serum bath on the ventricular strip. The amounts of the three salts in this solution were: sodium chloride, 0.6–0.7 per cent; calcium chloride, 0.026 per cent; potassium chloride, 0.03–0.04 per cent; a trace of sodium carbonate was sometimes added. This solution of the inorganic salts of blood will keep a heart strip alive and in condition to beat for a very long time, 72 hours and more. The inorganic solution does not keep the strip in as good condition as does blood or serum, but the parallelism between the two is very striking. The following experiment exhibits the close relation in the action of the above inorganic salt solution and of serum on ventricular strips.

Experiment 34, January 29, 1898. The apical third of the ventricle was cut into thin strips which were suspended in heart tubes while still saturated with blood. Strip *a* was immersed in pure serum; strip *d* in a bath of Ringer's mixture (0.6% NaCl, 0.026 $CaCl_2$, 0.04% KCl, and a trace of Na_2CO_3).

a. The serum strip gave a few beats when first suspended, but in a few minutes became quiet in a relaxed state. Single contractions occurred at long and irregular intervals during ninety-six hours, when the strip was changed to a bath of 0.6 per cent sodium chloride. Sodium chloride solution called forth the customary series of contractions.

d. The strip in Ringer's solution remained quiet in a relaxed state, giving only occasional single contractions during seventy-six hours. The contractions which were recorded during a portion of this time did not average more than one per hour. At the end of seventy-six hours the strip was changed to a bath of 0.6 per cent sodium chloride solution, and a rapid series of contractions was produced at once (see Fig. 4). While the maximal contractions of this series were only 0.5 cm. high, about one half the height of those of strip *a* in saline, still the series resembled a typical sodium chloride series in that the contractions rapidly declined in amplitude until the strip remained quiet in the diastolic state.

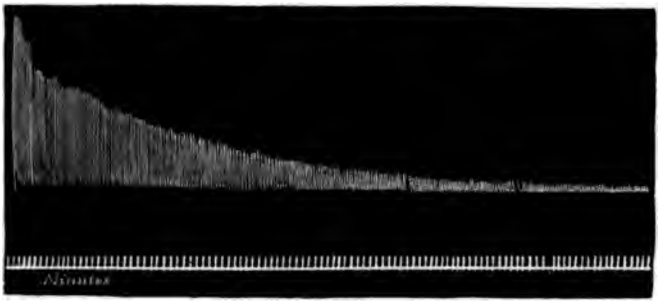

FIGURE 4. Experiment 34 *d*, January 29, 1898. Vertical magnification 4/1. Time trace marks minutes. To illustrate the effect of a 0.6 per cent bath of sodium chloride solution on a strip of terrapin's ventricle after seventy-six hours' suspension in Ringer's solution. When the sodium chloride was introduced, regular contractions began at once, as the above shows.

The following is a second example of the similarity of action between the solution of inorganic salts and pure serum: —

Terrapin 45, February 28, 1898. The apex of the ventricle saturated with blood was cut into thin strips and suspended in heart tubes, *a* in Ringer's solution, *b* and *c* in air. Strip *b* was moistened by occasional drops of Ringer's solution[1] (0.7% NaCl, 0.02% $CaCl_2$, and 0.03% KCl), and *c* with drops of serum.

Strip *a*, after a latent period of twenty minutes, gave contractions that were perfectly normal in character but very irregular in rate. The rate averaged one to four per minute. Neither *b* nor *c* contracted for thirty consecutive hours, but when moistened with sodium chloride solution both strips gave contractions that were complete and apparently normal in character.

This experiment again demonstrates that solutions of inorganic salts, as well as serum, are able to keep the ventricular strip in good condition, and that they, like serum, will not necessarily stimulate the strip to rhythmic contractions.

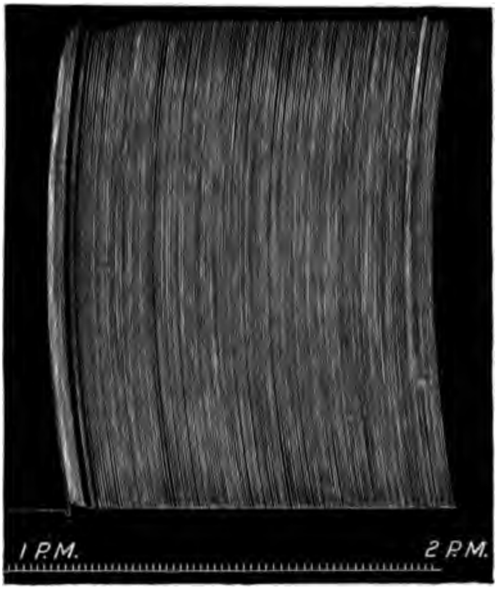

FIGURE 5. Experiment 42 *b*, February 14, 1898. Vertical magnification 4/1; time trace marks minutes. A ventricular strip from the apex of a terrapin's heart, moist with Ringer's solution, and contracting in a moist chamber. See protocol of Experiment 42. This strip, at the end of twenty-two hours' suspension in air, and still moist with Ringer's solution containing 0.7% NaCl, 0.026% $CaCl_2$, and 0.03% KCl, contracted only at long intervals, giving only sixteen contractions in the last two hours. When the strip was moistened with Ringer's solution, with the calcium chloride increased to 0.04%, the above beautifully regular contractions began and continued for one hour fifty minutes without interruption. Rate, four per minute. Compare with Fig. 6.

This experiment is also important in its bearing on another experiment to be given presently, Experiment 42. The fact that one heart strip immersed in a given inorganic solution beats while another strip from the same heart does not beat when in air saturated with water vapor, and moistened only occasionally with the same inorganic solution, indicates some process more favorable to the development of contractions in the presence of the liquid bath. This cannot be ascribed to any hindering effect due to the presence of the air. In fact, one would expect the better aëration of the tissue surrounded by air to facilitate the development of contractions rather than to delay contractions. This point is brought forward and emphasized at this time; for if strips be first washed out in sodium chloride solution, then bathed for a few minutes with Ringer's solution, and suspended in air, they may become as much as ten times more active than strips kept in a constant bath of the same solution.

The particular results to be expected when a fresh strip of ventricle containing the normal amount of blood is subjected to a bath of sodium, calcium and potassium chloride solution, so far as my experiments go, depend upon the relative proportions of the calcium and potassium chlorides. If these salts are in the proportions of 0.026 per cent calcium chloride to 0.03 per cent potassium chloride, a few good contractions at a very slow and irregular rate may result. If this ratio is changed by increasing the calcium or by decreasing the potassium, then the contractions are increased in frequency (Figs. 5 and 6). But if the calcium is diminished or the potassium increased, few contractions are developed, or none at all. Experiment 34 *d* gave fifteen contractions in the first forty-six hours. The solution used contained 0.6 per cent sodium chloride, 0.026 per cent calcium chloride, 0.04 per cent potassium chloride, and 0.003 per cent sodium carbonate.

If the muscle strip is cut from a heart previously irrigated with saline, or from one that has been subjected to the influence of some other artificial solution, the effect produced on the strip by Ringer's solution varies according to at least two factors: first the relative amounts of the calcium and potassium chlorides in the Ringer's solution, and second, the composition of the solution to which the strip has previously been submitted, together with the time the latter has acted.

Ringer's solution following sodium chloride solution. — A ventricular strip treated with sodium chloride, 0.6 to 0.7 per cent, until it ceases to contract, is stimulated to a strong and regular series of contractions when bathed in a suitable Ringer's solution. The amplitude and general character of the contractions of a strip revived in this way can only be compared with the contractions of a strip revived by serum or by serum diluted with saline. By moistening the strips with Ringer's solution, in which the proportion of calcium chloride is increased from time to time, strips may be kept beating for sixty hours and more after the apparent exhaustion in sodium chloride. In one experiment, No. 42, three companion strips were in this way kept contracting for fifty-one and a half, sixty-two, and forty-two hours.

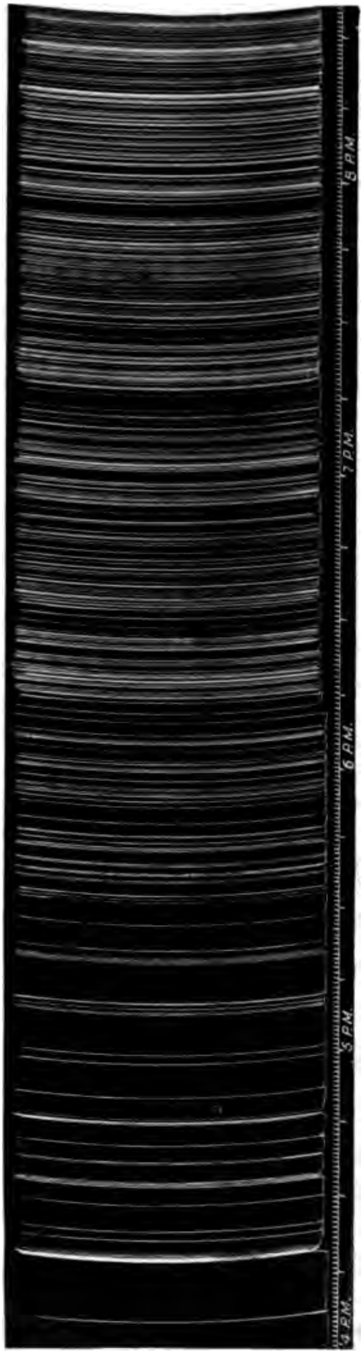

FIGURE 6. Experiment 42 *a*, February 14, 1898. Vertical magnification 4/1. Time trace in minutes. To illustrate the effect on a quiet strip of increasing the amount of calcium chloride in the Ringer's solution used. The curves are about one half the original size.

The ventricular strip from which this trace was obtained was from a terrapin's heart that had been washed free of blood with 0.6 per cent sodium chloride solution, then suspended in a Ringer's solution until a strong rhythm was established, and finally left contracting in moist air. The contractions were always full, but the rate became slower and more irregular, and ceased at eighteen hours after suspension. The strip was now moistened with stock Ringer's solution, then with a solution containing successively 0.03%, 0.04%, and 0.05%, and finally 0.06% calcium chloride. The last moistening occurred at the twenty-seventh hour of suspension. In twelve minutes after the last moistening contractions began again at an irregular rate. The contractions increased in frequency, and became fairly regular in four hours, as the above trace illustrates. See also Fig. 5.

In another experiment, 33 *a* and *d*, strips immersed in Ringer's solution contracted rhythmically and normally for twenty-seven hours, when the record was lost. After forty-two hours these strips were quiet, but apparently in good condition for contraction, as both strips were immediately revived, and gave fine contractions when changed to serum. The recovery in both these strips in serum closely resembles the recovery of contractions in strips treated with sodium chloride, and then changed to Ringer's solution or to extract of serum. This suggests a recovery due to the effects of the better combination of salts in the terrapin's own serum. At least, I am strongly inclined to believe that the revival in this case is due to the fact that serum contains the conditions or substances most favorable to the complete using up of the contractile material still in the strip.

If the strip is changed from sodium chloride to Ringer's solution while the contractions are small and regular in rate, the revival of complete contractions is very prompt. The contractions become maximal in a very few minutes, and the rate is often perfectly regular for a time. Afterward, however, the rate becomes slower and very irregular, a result perfectly analogous to the serum effect under the same circumstances.

On the other hand, if the contractions have entirely ceased in sodium chloride, and the muscle has remained in the solution from one to several hours, and then is suddenly subjected to a bath of Ringer's solution, the result is quite different. Under such circumstances the muscle is thrown into immediate fibrillation, from which it never recovers while in the Ringer's solution. This is apparently strictly a calcium effect. For if, instead of the ordinary combination of salts in the Ringer's solution, a solution containing a reduced amount of calcium is first applied to the strip and the calcium gradually increased to the normal amount, good contractions are produced and fibrillation avoided.

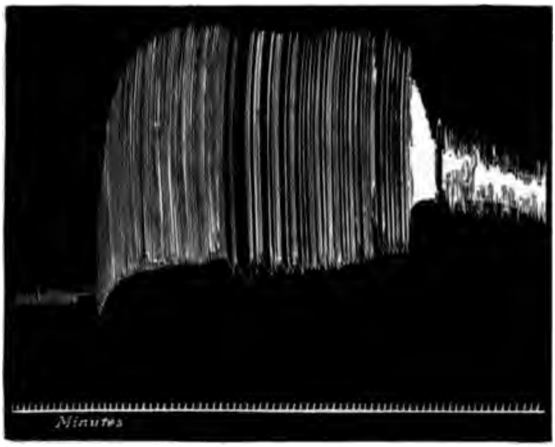

FIGURE 7. Experiment 46 *a*. March 2, 1898. Vertical magnification, 4/1. Time trace marks minutes. To illustrate the effect of a favorable combination of the salts in Ringer's solution in reviving contractions in a strip of the terrapin ventricle after its contractions in sodium chloride were reduced to a fraction of a millimetre in height. On immersing the strip of ventricle in Ringer's solution the contractions quickly increase in amplitude to a maximum, become irregular in rate after twenty minutes, and pass into fibrillation after about fifty minutes. Seven ninths the original size.

Muscle strips left in sodium chloride solution a long time invariably go into strong contracture when changed to ordinary Ringer's solution, whether they beat or not. If fibrillation results, the tonic shortening amounts to one third or even one half the amount of shortening previously produced in the same muscle during a normal contraction. If the saline strip is treated with a solution of sodium chloride and potassium chloride before the Ringer's solution is used, contracture is diminished and fibrillation delayed, though not necessarily avoided.

In certain experiments normal Ringer's solution following sodium chloride solution produced an excellent recovery for a time, but later the strips went into a state of fibrillation. Perhaps it would be better to describe this state as one of incoördinated contractions followed by fibrillation. Such strips while giving regular normal contractions in Ringer's solution suddenly show independent rhythm in two or three parts of the strip at the same time, and then gradually pass into fibrillation.

In a single example, a strip contracting in serum after repeated revivals from sodium chloride solution gave much larger beats when changed from serum to Ringer's solution (see Fig. 2 *a*). The height increased from 0.6 cm. to 1.05 cm. The rate, which was irregular both before and after the change, was increased from an average of 0.3 to 2.8 per minute. This effect followed at once, and is presumably due to the stimulating effect of the calcium.

Ringer's solution following a solution of sodium and potassium chloride. — A normal strip that has been kept from contracting by a solution of potassium in sodium chloride gives normal rhythmic contractions when the bath is changed to Ringer's solution. If the amount of potassium chloride in the sodium and potassium solution has been great, say 0.04 to 0.05 per cent, the contractions called forth by the bath of Ringer's solution may be at a very slow rate, or may appear in groups at a good rate, but with the groups separated by long periods of rest, during which the muscle remains in a perfectly relaxed state. Ringer's solution after a solution of sodium and potassium chloride is not followed by tone shortening, such as occurs after sodium chloride alone.

If a strip in a sodium and potassium chloride solution be transferred first to a Ringer's solution having a reduced amount of calcium, and later the calcium be increased, no contractions are developed until the amount of calcium in the Ringer's solution is increased to at least the normal amount found in blood.

Of the strips which were transferred to a bath of ordinary Ringer's solution after treatment with a solution of sodium and potassium chloride, two developed full normal contractions in from five to ten minutes, and after forty to sixty minutes each strip fibrillated. Presumably this result was due, in each case, to the strong stimulating effect of the calcium, which was fully counteracted by the potassium until the calcium had time to diffuse more completely into the strip.

EXTRACT OF THE EVAPORATED RESIDUE OF SERUM.

Terrapin serum was dried over a water bath, and the dried residue pulverized and extracted with distilled water. The filtered extract was evaporated to dryness and a second extract made and diluted to the original volume of serum. The second extract gave only a very faint color change when tested for proteid by the xanthoproteic test, and no proteid was detected by Millon's reagent. Careful testing for sugar with Fehling's fluid gave a questionable precipitate. This extract was compared with blood and with Ringer's solution in its reviving effects on the heart strip after saline treatment. The close similarity in the results is shown in the following protocol and the accompanying Fig. 8.

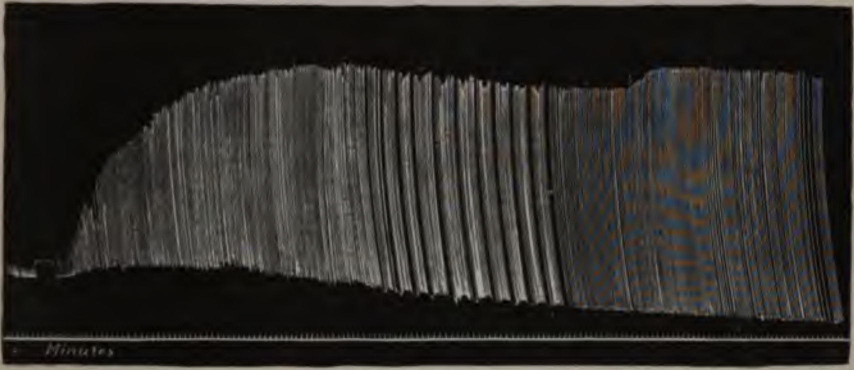

FIGURE 8. From Experiment 46 *c*, March 2, 1898; vertical magnification 4/1. Time trace marks minutes. To illustrate the effect of an extract of the dried residue of terrapin serum in reviving full contractions in a strip of ventricle that had almost ceased to contract in sodium chloride solution. Companion to the tracing shown in Fig. 7.

Experiment 46, March 2, 1898. Three ventricular strips cut from the normal heart were first immersed in saline until the contractions produced in each were reduced to 0.04 cm. and less in height. Strip *a* was transferred from saline to Ringer's solution (0.7% NaCl, 0.026% $CaCl_2$, 0.03% KCl). Strip *b* was transferred to serum, and *c* to an extract of the salts from the evaporated residue of serum.

Strip a. So soon as Ringer's solution surrounded the strip, contractions began and continued at a regular rate but rapidly increased in height with each successive contraction until in ten minutes they had increased from 0.04 cm. to 0.8 cm. The rate remained regular for twenty minutes, then became irregular for thirty minutes. After fifty minutes the strip gave a series of very rapid contractions and then went into fibrillation, from which it did not recover. (Fig. 7.)

Strip b. In serum; was accidentally lost.

Strip c. The extract of the salts of serum produced a rapid recovery of the amplitude, but not so rapid a recovery as that of strip *a* in Ringer's solution. The height increased in thirty minutes from 0.03 cm. to 0.9 cm. The rate for the first hour was slightly irregular, being from four to five per minute. After one hour in serum extract Luciani's periods appeared. There were ten of these groups in forty minutes. In every group the contractions began at a slow rate, increased to a maximum rate, then became slow again. A pause of from thirty to sixty seconds intervened between each two of the several groups. The rate following the groups was perfectly regular — two per minute — for several minutes. At the end of two hours and thirty-five minutes the contractions suddenly ceased entirely. With the exception of three single contractions the strip remained quiet in relaxation until transferred to 0.7 per cent saline, when it gave the customary saline series. The last contractions of the serum extract series were 1.03 cm. high. This series ended very much like that of a slightly diluted serum.

On the whole, the serum extract revived the strip *c* more normally, that is, more like serum, than did Ringer's solution in the case of strip *a*. The after effects were also more like serum, as shown in other serum experiments. Another experiment with serum extract, on strip *a* of Exp. 46, bears out this view. Serum extract was used on the strip when in fibrillation after long exhaustion, and at once produced increased activity. After seventy minutes the fibrillating strip relaxed suddenly, and began slow and irregular, yet apparently perfect contractions. The relaxation was, however, only partial in this case. This was the only instance in all my experiments in which I obtained this type of recovery from fibrillation with anything but pure serum.

ON THE WORK GIVEN OFF BY A CARDIAC STRIP AND THE SOURCE OF THE ENERGY OF CONTRACTION.

The amount of energy liberated by a heart strip bathed in an inorganic salt solution has been so great in many experiments as to raise the question as to the source of the energy-giving material.

In Experiment 33 *a* a fresh strip that had been bathed in 0.6 per cent sodium chloride until no further beats were produced was changed to a bath of Ringer's solution. It almost immediately gave contractions which were normal in general character, and higher and stronger than the largest contractions obtained from the strip while in the sodium chloride bath. The rate, however, was slow and irregular. The contractions of this strip in Ringer's solution increased in height and improved in rate, and in twenty-seven hours gave 1670 gramcentimetres of work. The record was lost from the twenty-seventh till the forty-second hour, at the end of which time the quiet muscle was transferred to serum. This partial record strongly suggests the view that the amount of energy developed by the muscle strip is greater than can be accounted for by the proteid constituents of the blood in the muscle. To test this question more thoroughly, Experiment 42, given in detail below, was tried.

Experiment 42, February 14, 1898. A terrapin's heart was washed as free as possible of blood by continuous irrigation with 0.7 per cent sodium chloride solution. The washing was facilitated by cutting the coronary veins and by gentle massage applied to the ventricle. Irrigation continued one hour, and at the end of that time the ventricle was still giving feeble contractions. Thirty minutes were consumed in preparing, weighing, and suspending three ventricular strips. They were, therefore, bathed in 0.7 per cent solution of sodium chloride an hour and thirty minutes. The strips in the heart tubes were next surrounded by a bath of Ringer's solution (0.7% NaCl, 0.026% $CaCl_2$, and 0.03% KCl) for thirty minutes, during which time strong contractions were established in each strip. The Ringer's solution was next drawn off, leaving the strips in moist air. They were occasionally moistened with a momentary bath or with drops of Ringer's solution. The three strips were kept thus in air fifty-one and a half, seventy-two and two thirds, and fifty hours respectively.

Strip a. This strip was kept moistened with Ringer's solution, of the composition given above, for seventeen hours twenty-five minutes. The contractions were comparatively rapid at first, about one half the total number of contractions occurring during the first seven hours. During this first period of suspension the rate slowly decreased, so that at the end of eight hours the strip was beating with an irregular rhythm and with a tendency to grouping. The rate temporarily increased after each remoistening of the strip. The amplitude began with 0.9 cm. actual shortening during a contraction, and slowly but steadily increased to 1.38 cm. in twenty-six hours; it remained above 1.0 cm. for forty-five hours. The contractions were apparently always complete whether the rate was fast or slow. The record was lost from thirty minutes past the tenth hour to fifteen minutes past the seventeenth hour of suspension in air. At the end of this time strip *a* was not contracting. Remoistening with the usual Ringer's solution produced no contractions. Ringer's solution with increased amounts of calcium chloride was then applied to the strip. During eight hours (the seventeenth to the twenty-fifth) the calcium chloride was increased successively to 0.03, 0.04, 0.05, and 0.06 per cent. With Ringer's solution containing 0.06 per cent calcium chloride, the strip again began to contract and continued to do so for twenty-six hours thirty minutes, with but one remoistening. The contractions, at first slow and irregular in rate, increased from forty the first hour (twenty-sixth of the experiment) to one hundred and sixty-one the sixth hour (thirty-second of the experiment). During the succeeding night the room temperature decreased to 4° C. and both rate and height of contractions were somewhat reduced, but both recovered again the next morning.

The forty-fifth hour after suspension in air the contractions began slowly to decrease in amplitude, reaching a zero in six hours, thirty minutes, *i. e.*, after a total time of activity in Ringer's solution of fifty-one hours and thirty minutes. The proportion of calcium chloride was again increased, but no contractions were produced even with 0.10 per cent.

A bath of horse's serum applied to the strip at fifty-one and a half hours was followed immediately by a series of contractions lasting for sixteen hours. The rate began with five per minute, but varied greatly at different times and the contractions were often quite incoördinated. In serum the height was 0.35 cm. at first, about one third that of the normal contractions of this strip, but decreased to zero in sixteen hours, *i. e.*, the sixty-seventh hour of the experiment. Minute contractions, 0.02 cm., were still obtained by diluted serum at the seventy-fourth hour.

Strip b. This strip was continued in air for seventy-one hours. For the first ten hours the record had all the general features of *a*, except that the contractions were at a somewhat slower and more irregular rate and were higher. Luciani's groups with well-marked "stair-case" occurred from the ninth to the twenty-second hour. Only sixteen contractions occurred from the twentieth to the twenty-second hour of suspension in air. Ringer's solution with the calcium chloride increased to 0.04 per cent was used to wet the strip at the twenty-second hour, and was immediately followed by tall, regular contractions for a period of over two hours. These contractions ceased quite abruptly. After a long pause — at the twenty-fifth hour — the strip was wet with drops of Ringer's solution containing 0.05 per cent calcium chloride. Immediately Luciani's groups appeared, with long intervening pauses. The groups became more and more frequent, and after three hours they passed into an irregular rhythm which continued twenty-five hours, thirty minutes. During the night the rate and height were both decreased by the low temperature, as in *a*. The calcium chloride was again increased in the Ringer's solution to 0.06 per cent, and at fifty-one hours, thirty minutes, the strip was given a two minutes' bath. The only effect was a slight increase in rate. Following the fiftieth hour of suspension the height of the contractions slowly decreased for twelve hours from 0.98 cm. to zero. Bathing the strip with Ringer's solutions containing 0.06 and 0.08 per cent calcium chloride, respectively, brought out contractions of good rate but only a millimetre in height (not recorded in the table below). At seventy-two hours strong electrical stimulation produced no observable contractions. At seventy-two hours, forty minutes, the strip was immersed in a bath of horse's serum, which produced contractions only 0.02 cm. to 0.03 cm. in height, *i. e.*, no recovery. The serum in this case, therefore, failed to cause an improvement in contractions. The experiment ended at the seventy-fourth hour of suspension in air.

Strip c. This strip in air contracted rapidly for a time but gradually became slower during twenty-four hours, forty-five minutes. Its contractions decreased in rate more gradually than in *a* or *b*, and Luciani's groups were not so prominent. Wetting the strip with Ringer's solution (calcium chloride 0.04 per cent) at twenty-four hours, forty-five minutes, was followed by an increase in the rate. After thirty hours, fifteen minutes, increase of calcium chloride to 0.05 per cent produced a slight increase in the rate. The height of the contractions slowly decreased from 0.69 cm. at the thirty-first hour to zero at the forty-second hour (see table below). The number of contractions for the last ten hours was very small. Further increase of calcium produced in each case comparatively rapid contractions, but no recovery of the height; thus, at the forty-second hour, the height of the contractions immediately following calcium chloride solution 0.06 per cent was 0.14 cm.; at the forty-fourth hour, following 0.08 per cent calcium chloride solution, it was 0.18 cm.; and at the forty-sixth hour, minute contractions were observed after 0.1 per cent calcium chloride. At the fiftieth hour the strip was given a bath of terrapin serum which produced contractions only 0.15 cm. high. No strong full contractions resulted.

I have made the amount of work given by these three strips of ventricular muscle while contracting automatically in a purely inorganic diet the basis of a series of calculations, in order to express the results in a way that admits of comparative study.

TABLE IV.

Tabulated results of Experiment 42 *a*, *b*, and *c*.

Time of suspension in air.	Number of contractions per hour.			Mean height of contractions during the hour = actual shortening of the muscle in centimetres.			Work done during one hour expressed as gramcentimetres.		
hour	a.	b.	c.	a.	b.	c.	a.	b.	c.
0-1	512	305	440	0.90	1.30	1.00	461	396	440
1-2	564	265	420	0.90	1.40	1.02	508	371	430
2-3	440	200	280	0.96	1.47	1.17	422	294	330
3-4	394	166	343	1.00	1.50	1.20	394	249	292
4-5	261	115	244	1.12	1.50	1.20	292	173	293
5-6	220	82	235	1.12	1.50	1.30	246	123	305
6-7	150	116	233	1.10	1.55	1.30	165	180	303
7-8	145	127	190	1.10	1.57	1.30	160	200	247
8-9 (12 P. M.)	98	44	114	1.15	1.57	1.37	113	69	157
9-10	100	64	110	1.15	1.60	1.40	115	102	154
10-10½	17	21	58	1.16	1.60	1.38	20	34	80
10½-17¼[1]									
17¼-18	10	93	45	1.20	1.62	1.40	12	151	71
18-19	0	87	85	0	1.62	1.40	0	141	119
19-20	0	95	82	0	1.62	1.40	0	154	115
20-21 (12 M.)	18	9	82	1.30	1.50	1.42	23	13	117
21-22	22	7	84	1.30	1.50	1.40	25	11	118
22-23	7	165	60	1.32	1.57	1.32	9	260	80
23-24	0	190	33	0	1.59	1.22	0	300	40
24-25	3	44	21	1.30	1.59	1.20	4	70	25
25-26	40	40	20	1.38	1.57	1.10	55	63	22
26-27	45	16	32	1.38	1.58	1.05	62	25	34
27-28	110	34	35	1.38	1.57	0.95	152	54	33
28-29	115	98	78	1.35	1.58	0.87	155	154	68
29-30	138	50	129	1.33	1.57	0.70	183	79	90
30-31	161	60	45	1.28	1.58	0.69	205	95	31
31-32	120	67	56	1.28	1.50	0.65	153	100	36
32-33 (12 P. M.)	80	64	18	1.22	1.45	0.60	98	93	11
33-34	59	38	20	1.20	1.38	0.50	71	52	10

[1] Record lost for six hours, forty-five minutes.

TABLE IV. — *Continued.*

Time of suspension in air.	Number of contractions per hour.			Mean height of contractions during the hour = actual shortening of the muscle in centimetres.			Work done during one hour expressed as gramcentimetres.		
hour	a.	b.	c.	a.	b.	c.	a.	b.	c.
34–35	38	33	16	1.15	1.30	0.45	44	43	7
35–36	24	29	16	1.10	1.25	0.40	26	36	6
36–37	23	19	7	1.10	1.20	0.04	25	23	3
37–38	23	58	3	1.05	1.20	0.03	24	70	1
38–39	28	32	6	1.10	1.22	0.02	31	39	1
39–40	27	33	4	1.10	1.22	0.02	30	40	1
40–41	32	46	7	1.11	1.22	0.01	36	56	1
41–42	60	48	4	1.05	1.20	0.01	63	58	0
42–43	57	60	14	1.00	1.05	0.01	57	63	2
43–44	69	60	13	1 00	1.08	0.01	69	64	1
44–45 (12 M.)	88	34	19	0.98	1.05	0.02	86	36	4
45–46	80	37	28	0.90	1.05	0.01	72	39	3
46–47	83	37	23	0.80	1.05	0	66	39	0
47–48	112	37	50	0.70	1.02	0	78	38	0
48–49	154	42	75	0.55	1.00	0	85	42	0
49–50	78	42	trace	0.25	0.98	0	20	41	0
50–51	10	42		0.05	0.82		0	35	
51–52		16			0.75			12	
52–53		37			0.70			12	
53–54		40			0.60			26	
54–55		44			0.60			24	
55–56		55			0.62			34	
56–57 (12 P. M.)		48			0.60			29	
57–58		42			0.52			22	
58–59		45			0.45			20	
59–60		34			0.22			8	
60–61		34			0.07			2	
61–62		16			0.05			1	
Total	4817	3762	3877				4915	4958	4061

The strips of muscle were weighed as carefully as possible before suspending them, and also at the close of the treatment with inorganic salts. At each weighing the moisture was removed as nearly as possible to the same extent by draining on glazed porcelain or on glass. The strips were then weighed between watch crystals. The results of all experiments where double weighings were made show a loss of weight. This fact is of significance, although I have not made a sufficient number of experiments for quantitative estimates from this standpoint.

TABLE V.

Weight of muscle strips at the beginning and end of treatment with Ringer's solution.

Number of the experiment.	Weight before treatment with Ringer's solution.	Weight after treatment with Ringer's solution.	Time of suspension in Ringer's solution.	Loss.
42 *a*	0.321 grams	0.196 grams	51½ hours	0.125 grams
42 *b*	0.283 "	0.231 "	72 "	0.052 "
42 *c*	0.340 "	0.232 "	50 "	0.108 "

The figures in this table, which express the weights before the use of Ringer's solution, are of the greatest importance for the present consideration. The three strips used gave respectively 4915, 4958, and 4061 gramcentimetres of recorded work. If this work be converted into its heat equivalent it may then be compared with the heat equivalents of the possible sources of energy-giving material. One gramcentimetre of work equals 980 ergs; one calorie equals 4.2×10^7 ergs; one gramcentimetre therefore equals $0.00002\frac{1}{3}$ calories.

The total heat of oxidation of one gram of proteid and of one gram of cane-sugar varies according to the determinations of different investigators. Stohman gives for beef (fat free) 5641, for urea 2465, and for cane-sugar 3959 calories respectively. Danilewsky gives for fibrin 5772, urea 2537, and for cane-sugar 4176 calories. Rubner gives for muscle extracted with water 5778, and for urea 2523 calories. If the oxidation equivalent of the urea formed as a result of the metabolism of one gram of proteid, *i.e.*, one third of a gram of urea, is deducted, it may be assumed that, in round numbers, the average oxidation energy of one gram of proteid or one gram of sugar available for muscular metabolism is 4890 calories for proteid, or 4000 calories for carbohydrate.

The amount of work recorded by the three strips is the equivalent of the oxidation of 0.0000233, 0.0000235, and 0.0000193 grams of proteid or of 0.0000287, 0.0000289, 0.0000234 grams of carbohydrate respectively. These amounts represent the oxidation equivalents if all the energy appears as work, whereas it is well known that only a small fraction of the total energy of metabolism can be utilized as work. Gaule,[7] 1878, computed that, at least, not less than eight per cent of the energy of contraction in the frog's heart may be recorded as work. The well-known experiments of Fick upon striated muscles show that under the most favorable conditions twenty per cent may be regarded as a maximum yield in work. In the cardiac strip, it must be remembered that many of the fibres are cut across so that their contractions are lost, while others may by their contractions oppose those which exert a direct pull on the lever. It is, therefore, a liberal estimate to assume that fifteen per cent of the energy of oxidation may take the form of work during contractions of the ventricular strip, and that not more than two-thirds of this,— ten per cent of the total energy, is recovered on the record. These facts are arranged for comparison in the following table: —

TABLE VI.

Number of the experiment.	Work recorded.	Mechanical equivalent in heat.	Total oxidation equivalent in proteid.	Equivalent in proteid of metabolism if 10% is recovered as work.
42 *a*	4915 gr. cm.	0.11468 calories	0 0000233 grams	0.000233 grams
42 *b*	4958 "	0.11569 "	0.0000235 "	0.000235 "
42 *c*	4061 "	0.09312 "	0.0000193 "	0.000193 "

The average amount of serum-albumin in the serum of this species of terrapin during the winter season, as determined by Howell[21] in 1884, is 0.69 per cent. If the energy of the heart muscle is supplied by the metabolism of serum-albumin, as Kronecker holds, the amount of blood required to supply the necessary albumin for each of the three strips mentioned above must have been, respectively, 11.5, 13.1, and 9 per cent of their initial weights. But the percentage of blood in the entire body (determined upon mammals) is only 7.7 per cent, a large proportion of which is contained in the great vessels. The percentages exhibited in the following table reduce the question to an absurdity in so far as considering serum-albumin as the source of the motor energy of the washed strips of the terrapin's heart is concerned.

TABLE VII.

Number of the experiment.	Work recorded.	Equivalent in the metabolism of serum-albumin of	Weight of serum necessary to supply the serum-albumin.	Initial weight of the muscles.	Proportion of serum in the muscles necessary to account for the work, etc.
42 *a*	4915 gr. cms.	0.000233 grams	0 0338 grams	0.321 grams	10.5%
42 *b*	4958 "	0.000235 "	0.0340 "	0.283 "	12.0%
42 *c*	4061 "	0 000193 "	0.0280 "	0.340 "	8.2%

The amount of paraglobulin in the blood of this terrapin is, according to Howell, comparatively large, 4.66 grams per 100 c.c. The equivalent of the above work may be expressed in terms of paraglobulin (Table VIII.).

TABLE VIII.

Number of the experiment.	Work recorded.	Equivalent in the metabolism of paraglobulin.	Weight of serum necessary to supply the paraglobulin.	Initial weight of the muscles.	Proportion of serum in the muscle strips necessary to supply the paraglobulin.
42 *a*	4915 gr. cms.	0.000233 grams	0 0050 grams	0.321 grams	1.6%
42 *b*	4958 "	0.000235 "	0.0050 "	0.283 "	1.8%
42 *c*	4061 "	0.000193 "	0 0041 "	0.340 "	1.2%

When it is remembered that the heart was irrigated with 0.7 per cent sodium chloride solution for one hour, and that the washing was facilitated by the contractions of the ventricle and by massage during this time, then the possibility of there being even this percentage of blood left in the capillaries or in the meshes of the tissues seems improbable.

The percentage of sugar in systemic blood varies from 0.1 per cent to 0.15 per cent. On this basis it would be utterly impossible to account for the work, assuming that the energy came from the consumption of sugar furnished by the serum still left in the strips.

The more rational view applicable to this case is that the heart has stored material in its cells, and that the contractions of the isolated ventricular strips are at the expense of this material. The voluntary muscles are admittedly able to contract at the expense of stored contractile material. Why, therefore, should this function be denied to cardiac muscle?

The above experiment seems to demonstrate, beyond doubt, that *the terrapin's heart contracts at the expense of an antecedent contractile substance stored up in its own tissue.*

The amount of work developed by the heart strips under the influence of sodium chloride solution, or, in fact, any of the inorganic solutions used in these experiments, is perfectly represented by the comparison of the height and rate of contractions as discussed throughout this paper under the various headings, since the load was kept uniformly at one gram with all the levers used in the experiments quoted.

EXHAUSTION OF HEART MUSCLE.

The word "exhaustion" as applied to the heart has been used to express many different states. At the present time it can no longer be employed without specifying the condition to which it applies. Before defining its use in this section, it will be interesting to sketch, briefly, its use in the literature on the subject.

In 1874 Kronecker and Stirling[3] found that a frog's heart filled with 0.6 per cent sodium chloride solution soon ceased to beat, and could be made to beat again only on the introduction of pure or diluted serum. They considered this condition brought on by sodium chloride solution one of exhaustion. From their experiments they were led to the conclusion that the heart in its contractions used material obtained directly from the blood. According to the view proposed by Kronecker and Stirling at that time, the frog's heart is exhausted in sodium chloride solution because the material of the blood necessary to each contraction is washed out.

Investigations tending to support and develop Kronecker's views were made by Stiénon,[6] 1878, Martius,[9] 1882, Kronecker and Popoff,[12] 1887, and White,[17] 1896.

In 1878 Gaule[7] showed that a heart which is quiet in sodium chloride could be made to contract for several hours by using a solution of alkali-saline, and that in this latter solution it gave off as many as one thousand contractions. This remarkable result can be explained, he says, only on the supposition that the muscle of the heart has the antecedent material which it uses in contractions stored up in its substance. When a heart will no longer beat in renewed alkaline sodium chloride solution, according to Gaule, it can be revived only by feeding it with blood. Apparently, according to his view, a heart is exhausted when its store of contractile material is used up.

In 1882 Martius,[9] working under the direction of Kronecker, ascribed the beneficial effect of alkali-saline not to a more complete utilization of stored material in the heart cells, as Gaule supposed, but to the fact that the alkali, by combining with carbon dioxide, prevented asphyxiation of the contractile tissue, and thus permitted a more complete utilization of the remnants of blood still in the interstices of the heart. Only when this material is used up is a heart truly exhausted. Martius stated that such a heart could be revived only by the use of substances like blood, serum, lymph, *i.e.*, substances containing serum-albumin, and he concluded that serum-albumin was the particular element in the blood used by the cardiac muscle in contraction.

In 1883 and 1885 Ringer[10] gave a new interpretation to the term "exhaustion," when he said "calcium salts are necessary for the proper contractions of the heart, yet they must be antagonized by potassium salts." According to this idea, a heart fails to contract in sodium chloride or in alkali-saline, because it is not supplied with the necessary calcium and potassium salts. Ringer's standpoint was still further emphasized by Howell and Cooke[16] in 1893, who showed that hearts that had ceased to beat after abundant irrigation with saline or alkali-saline could be revived and kept in normal contractions for long periods when supplied with Ringer's solution or with extracts of milk, blood, or gastric juice that contained only traces of proteid.

When in 1896 White[17] wrote in support of Kronecker's view he was compelled to take refuge in an hypothesis which attempts to overthrow the most painstaking work by one word. This hypothesis assumes that the heart may beat on infinitesimal, and, one may add, indetectible quantities of serum-albumin, and that it is almost if not quite impossible to completely remove all traces of serum-albumin from the muscular spaces of the heart.

White said that by the methods used by Merunowicz, Aubert, Martius, and other investigators, "it was impossible to completely wash out a heart." He held, therefore, that their conclusions were unfounded. He said that a heart must be irrigated through the most improved perfusion cannula, that it must be irrigated by successive solutions of sodium chloride, alkali-sodium chloride, and Ringer's solution until it is quiet. Only when it no longer responds to any of these solutions is it free from serum-albumin and truly exhausted. This is, however, the familiar argument in a circle, since the conclusion proceeds directly from the hypothesis assumed in the beginning. White also committed the grave error of not using the most favorable combination of salts in Ringer's solution, and, therefore, did not secure completely washed-out hearts in the sense in which he uses the term. By his own process of reasoning his results must be placed in the same category as those of the earlier investigators. In four examples that he gives, the hearts were exhausted after a total time of irrigation of: 1 hour, 25 minutes, 5 hours, 30 minutes, 9 hours, 15 minutes, and 4 hours, 45 minutes.

In this laboratory, in recent experiments upon the whole heart of the frog, the results of which will be published later, it has been observed that after the heart has ceased to beat upon the Ringer's mixture used by White it may still beat, and beat well, upon the mixture generally employed in this paper (0.7% NaCl, 0.026% $CaCl_2$, and 0.03% KCl). Moreover, with many hearts, although not with all, it has happened that after they have ceased to beat upon this last mixture the gradual increase of the amount of calcium salts in the Ringer's solution called forth new beats for a considerable period, an effect also obtained on heart strips, as previously described in this paper.

From the above review it will be seen that the term "exhaustion," as applied to the heart, either expresses states in which the inorganic salts necessary to the contractions of the heart are removed or disturbed in their relations, or it expresses states in which the antecedent organic contractile material is consumed or removed. In the first group may be included as many conditions of exhaustion as there are combinations of the inorganic salts that will not support contractions. That the series of beats obtained with any solution should disappear by successively smaller contractions, as they do in a heart filled with sodium chloride solution, is non-essential. In the second group must be included, on the one hand, Kronecker's view, that exhaustion signifies a lack of sufficient free serum-albumin surrounding the muscle tissue to support contractions, and, on the other, Gaule's view, that exhaustion means a lack of antecedent contractile material stored in the cardiac tissue.

It seems to me it would be better to restrict the application of the term "exhaustion" to states of the cardiac tissue itself, and to designate in some other way all those conditions which imply the presence or absence of some substance or substances in the surrounding blood or artificial fluid. My own experiments are full of examples that demonstrate the inefficiency of sodium chloride solution to produce a true exhaustion in the ventricular strip. Time after time the sodium chloride solution series has been almost exactly duplicated on the same strip after an intervening recovery, due to a bath of solutions of inorganic salts alone. It is admitted that an inorganic diet cannot serve directly as a source of energy. My experiments give no convincing proof of the ability of the isolated muscle to use even the organic material of serum. In Experiment 42, given above, strips *a*, *b*, and *c* were made to contract incompletely with serum after they could no longer be aroused by Ringer's solution; but *b* gave only the slightest movements when treated with serum, and in both *a* and *c* the revival of contractions due to serum treatment did not last as long as did the contractions of *b*, still moist with the inorganic salt solution. It is possible, therefore, that the serum effect on *a* and *c* was due entirely to the more favorable relation of the inorganic salts, and produced only a more perfect exhaustion of the contractile material stored in the muscle cells of the strips.

The particular type of dying out of the contractions given by the strips in Experiment 42, quoted above, in which, after many hours, the amplitude of contractions slowly but steadily decreases to a zero from which no good recovery can be obtained, has occurred so often that it may be expected with confidence whenever muscular strips of the terrapin's ventricle are treated with any solution favorable to the development of continuous rhythmic contractions. In fact, in the very first experiment of this investigation, a heart strip, after seventy-two hours' continuous contraction, ceased in the same way as strips *a* and *b* of the above experiment. The strip of this first experiment was suspended in a muscle moist-chamber, and was made to begin its series of contractions by moistening it with serum much diluted with 0.6 per cent sodium chloride.

This type of dying out of the contractions I take to indicate a using up of the organic contractile material in the muscle. The completeness of exhaustion in this sense depends upon whether the muscular strip is subjected to the most favorable relation of the inorganic salts of the blood, especially of the sodium, potassium, and calcium salts. Whether or not the isolated strip is capable of being nourished, that is, of utilizing the stored energy of the organic constituents of the liquid in which it is immersed, is an independent question.

SUMMARY.

1. A bath of normal serum will not keep a strip from the apex of terrapin ventricle in contraction, although it keeps it in good condition for contraction for three or four days. By slightly increasing the amount of calcium chloride in the serum regular contractions may be produced.
2. An artificial mixture of sodium, potassium, and calcium salts in the proportions in which they exist in serum acts like serum, in that it does not produce a continuous series of rhythmic contractions, but sustains the cardiac strip in good condition for contraction, for at least three days. For the terrapin's heart this proportion is approximately 0.7 per cent sodium chloride, 0.026 per cent calcium chloride, and 0.03 per cent potassium chloride.
3. Sodium chloride will produce and sustain contractions for a short time only, and the series of contractions presents the appearance of fatigue. This appearance of fatigue indicates only the removal of the inorganic salts necessary to contraction, and is not an exhaustion of the contractile substance of the muscle.
4. Calcium salts in isotonic solutions of sodium chloride stimulate the cardiac strip to increased rhythm and final permanent contracture.
5. Potassium chloride in isotonic solutions of sodium chloride prevents contractions, and keeps the ventricular strip in a state of relaxation.
6. There is an optimum ratio of the potash, calcium, and sodium salts in isotonic solution most favorable to the development and maintenance of the contractions in the ventricular strip. For the apex of the ventricle of the terrapin this proportion is sodium chloride 0.7 per cent, calcium chloride 0.04 or 0.05 per cent, potassium chloride 0.03 per cent, if the strip is fresh and filled with blood.

If the strip is from a heart that has been washed with 0.7 per cent sodium chloride, the proportion of salts given in conclusion 2, above, is the most favorable to the maintenance of contractions. The rhythm in the spongy ventricular strip is rarely perfectly regular in this solution.

7. Complete exhaustion of the contractile substance in the heart of the winter terrapin is brought about by the use of inorganic salt solution only after thirty to seventy-two hours' continuous rhythmic activity, or by seventy-two to one hundred hours' suspension, if the activity has been slight.

8. Cane sugar and urea in isotonic solutions do not produce rhythmic contractions in the isolated strip. Dextrose in isotonic solution throws the strip into strong tone and may produce an imperfect series of contractions.

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THE COÖRDINATION OF THE VENTRICLES.

BY W. T. PORTER.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

ALL who have studied the mammalian heart in action have been impressed by the admirable synchronism of the contractions of the two ventricles. So perfectly do the right and left hearts work together that the observer is led at once to the opinion that their unison must be enforced by some master mechanism, such as a group of ganglion cells. The presence of nerve cells in the heart, and especially their presence in the basal part of the ventricles, lends color to this view, and we cannot therefore be surprised that the coördination of the thousands of muscle fibres of the ventricle into one harmonious contraction, and especially the synchronism of the contractions of the two ventricles, differing as they do in their muscular power and in the work required of them, should be believed to be a function of the nerve cells to which so great a share in the regulation of the heart-beat is ascribed.

I have demonstrated in a previous investigation[1] that at any rate the coördinated contraction of a single ventricle is not dependent on the influence of nerve cells. It was found that the mammalian ventricle might be kept beating for a very long time if supplied with blood through a cannula tied into the coronary artery. Ventricles cut entirely away from the auricles and thereby for the first time completely isolated, were kept contracting thus. Even any portion severed from the remainder will beat when fed with blood through the artery supplying it. The inevitable conclusion from these experiments is that *the coördination mechanism, whatever it may*

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[1] PORTER: Journal of experimental medicine, 1897, ii, p. 391.

be, is present in all parts of the ventricle. The same method was then used to determine whether the coördination of the ventricle was dependent on nerve cells. A cannula was tied into the ramus descendens of the left coronary artery not far from the apex of the left ventricle; and that part of the apex of the ventricle which could be fed through the cannula was excised and perfused with blood. In a few moments regular and strong contractions set in. An hour and forty minutes later the apex was still beating, when the experiment was broken off. In another experiment the apical ten millimetres of a dog's ventricle was kept in coördinated rhythmical contraction during several hours. The contractions thus secured were not the rhythmic response of the ventricle to an abnormal constant stimulus due to changes in the perfusion blood or to the 0.8 per cent sodium chloride solution with which the blood was frequently diluted; the contractions are usually more vigorous on undiluted blood than on that containing normal saline solution. They occur also when the piece of apex is fed in the living animal with the wholly normal blood supplied to the rest of the heart. Thus a branch of the ramus descendens in the apical region on the anterior surface of the left ventricle was freed from its bed and an oval piece of the ventricular wall supplied by this branch was separated from the remainder of the ventricle by an incision that ran parallel to the surface of the heart about four millimetres beneath the pericardium. The isolated piece remained in connection with the ventricle only by a strip of pericardium several millimetres long, which supported the artery and its accompanying veins, and was absolutely free of muscular tissue; long-continued, spontaneous, coördinated contractions were obtained from this piece, the rate being somewhat slower than that of the whole heart. As the apical portion of the ventricle contains no nerve cells,[1] these experiments furnish conclusive proof that nerve cells are not essential to the coördinated contractions of the ventricle.

The problem of the dependence of the coördination of a single ventricle upon nerve cells having been thus solved, the next question to be attacked is whether the remarkable synchronism of the right and left ventricle is also independent of nerve cells.

I was at first in doubt whether the apical halves of both mammalian ventricles could be made to beat together, because the arterial supply is such that both ventricles cannot be fed very well through a cannula in the ramus descendens of the left coronary artery, which is the only thoroughly practicable branch to the apex. My doubts were entirely dispelled by the following experiments.

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[1] This fact has been recently once more demonstrated by the careful work of SCHWARTZ: Archiv für mikroskopische Anatomie, 1898, lviii, p. 63.

Experiment November 14, 1898. A dog anæsthetized with morphia and ether was bled from the left carotid artery, the blood defibrinated, and filtered through glass wool. Meanwhile, warm 0.8 per cent sodium chloride was allowed to flow into the right jugular vein. After a short interval the dog was bled again from the carotid artery, and the blood defibrinated as before. The heart was now taken out, a cannula tied in the ramus descendens at the beginning of the apical half of its course, and the apical half of the heart severed from the basal half with a sharp amputating knife. The apex of the heart is formed chiefly by the left ventricle, so that the transverse incision just described includes strictly speaking the apical half of the left ventricle, but only the apical fourth of the right. Fibrillation set in as soon as the section was made, but probably disappeared before perfusion was begun; for when the cannula was connected with a reservoir containing the defibrinated blood at a temperature of 36° C. and under a pressure of about 100 mm. Hg., strong, fully coördinated contractions of the whole preparation began. The whole mass beat together as one piece; the synchronism of the right and left halves was perfect. Fifteen minutes later, the gas flame under the water tank surrounding the blood-reservoir having been left too high, the temperature of the blood rose to nearly 40° C., and the heart began to fibrillate. The supply of blood was then interrupted, in the hope that asphyxia would cause the fibrillation to disappear, as is often the case. Asphyxia failing, recourse was had to cold normal saline solution.[1] On reducing the temperature of the heart, the fibrillation gave place to coördinated contractions. When the warm blood was suddenly turned on again, the preparation at once shortened visibly and began to fibrillate.[2]

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[1] PORTER: This journal, 1898, i, p. 71.

[2] During the past two years I have repeatedly observed that the tonus of the ventricle was apparently greatly increased at the onset of fibrillation. The same fact has long been noted by students of the effect of electrical stimulation on the heart. Repeated experiments on the isolated ventricle have shown me that this shortening is an invariable, or almost invariable accompaniment of fibrillation, and that coördinated beats do not return unless the extreme, apparently tonic contraction is considerably lessened. It is evident that during fibrillation not all of the contractile units are in action at the same time or in the same way. Either the continued shortening observed is (1) a true tonus, dependent perhaps on the excessive action of contractile substance of the sluggish sort (Grützner), or (2) it is due to an abnormal, long-continued, tonic contraction of a number of the cardiac muscle fibres. The appearance of such a spasm at several points might break up the normal conduction of the contraction wave by interposing here and there regions, the intense spasmodic contraction of which would block the passage of the contraction wave, just as it is blocked experimentally by

Experiment November 15, 1898. A preparation of the apical half of the ventricles of a dog's heart was made as described in the preceding experiment. On perfusion the heart at once began to beat very strongly. The coördination of the right and left side was perfect.

It may be remarked that the contractions thus obtained are much more vigorous than those given by smaller pieces of the ventricle. The force of the beat is indeed astonishing. With each stroke blood shoots from the margins of the strip a considerable distance into the surrounding air. No better method for demonstrating the compression of the intramural vessels in systole could be desired.[1]

The experiment, just cited, together with similar ones on November 16 and 17, demonstrate conclusively that *the synchronism of the mammalian ventricles is not dependent on nerve cells.*

Another problem now arose. Is the coördination of the ventricular contractions in warm-blooded animals accomplished by the muscular elements alone or by the aid of the nerve fibres with which all parts of the heart are so plentifully supplied? Engelmann's well-known experiment, slitting the frog's ventricle by a zigzag cut running to and fro across the line taken by the nerves in such a way that the nerves entering the upper end of the zigzag strip are in all probability severed by one of the cuts before reaching the end of the strip, has answered for the batrachian heart in favor of the muscular fibres. A like experiment can be done on the mammalian heart, and with the same result. Contrary to long-established belief the mammalian ventricle perfused with blood diluted with sodium chloride solution, or wholly undiluted, as a rule continues to beat in spite of extensive incisions. In my paper "On the Cause of the Heart-beat,"[2] it is recorded that on March 22, 1897, the isolated heart of a cat was made to resume its regular contractions by feeding the coronary arteries through the aorta with defibrinated cat's blood. While the heart was

squeezing the muscle fibres together with a clamp, and would leave the remaining muscle fibres dissociated and in confusion. The second of the two above explanations is the more probable, in my judgment, but the evidence which I have collected, while more than sufficient to justify the making of an hypothesis, is not yet wholly decisive. Dr. C. C. Stewart has suggested to me that the shortening may be explained by the evident failure of all the muscle fibres to contract together; the contraction of some of the very numerous fibres must almost necessarily though by chance coincide, and this continuous coincidence would give the appearance of continuous, tonic contraction.

[1] PORTER: This journal, 1898, i, p. 145.

[2] PORTER: Journal of experimental medicine. 1897, ii. p. 392.

in full play the right ventricle was separated from the left and the interventricular septum cut out. Both right and left ventricles continued their rhythmic contractions. Two days thereafter, a cat's heart was removed from the body and fed with cat's blood diluted with 0.8 per cent sodium chloride solution. Coördinated contractions were secured. The right ventricle was then almost wholly separated from the left, yet both ventricles continued to beat in unison. The interventricular septum was now extirpated, but the synchronism of the ventricles was not disturbed. On slitting each ventricular portion from apex to base, the incisions being carried almost to the auricles, the four pieces beat synchronously. Similar incisions made in the frog's heart have been recently described in the interesting communication of Professor von Vintschgau.[1] The mammalian heart, however, is better adapted to these studies than the heart of the frog because of its large size, and especially because the nutrition of the frog's ventricle is suspended by opening the ventricle, while the nutrition of the mammalian ventricle may be carried on through the coronary arteries without the aid of the ventricle.

Persuaded by the observations of 1897 that the mammalian ventricle would continue to beat even under the conditions of Engelmann's procedure, the following experiments were performed.

Experiment November 16, 1898. The apical half of a dog's heart was perfused with blood through a cannula in the ramus descendens, as described above. Admirable coördinated contractions synchronous over both ventricles were secured. The ventricles were slit from the apex towards the base into many pieces, each supplied by a branch of the descendens artery. All contracted synchronously. The junction of each severed piece with the rim of uncut muscle at the base of the preparation was narrowed by transverse cuts until only a small neck remained, so that each piece communicated with the other pieces only by this narrow bridge. The muscular tissue remaining in the bridge was less than a square millimetre in cross section. Coördinated contractions were still synchronous in all the pieces. Some of the bridges were divided, leaving these pieces hanging from the basal ring of the preparation only by the nutrient artery and its connective tissue. Each piece thus separated beat with a rhythm of its own and no longer contracted synchronously with any other portion. Zigzag cuts were now made in several strips, but in each case they failed to prevent synchronous contraction; so long as a slight muscular connection was preserved the contractions of the partially severed portions remained synchronous.

[1] Von VINTSCHGAU: Archiv f. d. ges. Physiol., 1898, lxxiii, p. 381.

Experiment November 17, 1898. As in yesterday's experiment, good co-ordinated contractions were obtained in the apical half of the dog's heart, and the ventricles slit from the apex toward the basal portion of the preparation into long pieces, each of which contained a branch of the coronary artery. One of these long strips was now cut into three parts by three pairs of incisions; one cut of each pair was carried from the left margin of the strip nearly to the nutrient artery, the other at the same level, from the right margin nearly to the artery; the three portions were thus left united by the nutrient artery, connective tissue, and a few shreds of muscular tissue. All three beat in unison. The muscular tissue joining the uppermost to the middle piece of the three was now divided. Immediately the middle and the lowest piece, thus deprived of all muscular connection with the remainder of the ventricle, began to beat with a rhythm of their own, but still in unison with each other. The uppermost piece on the contrary continued to contract synchronously with the remainder of the heart.

When one piece of several connected together merely by the nutrient artery and a very small muscular bridge was thrown into fibrillation by rapidly repeated induction shocks, all the pieces at once fibrillated (in other hearts, this did not always succeed, probably because the power of conduction in the bridge was too much reduced), but when adjoining pieces were connected only by the artery and connective tissue and not by muscle fibres fibrillation was no longer transmitted from one to the other.

Engelmann's zigzag incisions were also tried on this heart, with complete success; synchronous contractions were observed in all the parts, weakened indeed by the interference with the blood-supply but still unmistakably present.

The fact that an extraordinarily small muscular connection between two pieces of mammalian ventricle will suffice to maintain the synchronism of the two was confirmed by numerous trials on fifteen additional animals, three dogs and twelve cats. There can be no doubt that the mammalian and the frog heart are alike in that a very small bridge of cardiac tissue suffices to carry the excitation wave from one part of the heart to the neighboring parts. It is in the highest degree improbable that in all of the numerous instances cited here the heart nerves should take the extraordinary recurrent and roundabout course necessary for them to reach all parts of the zigzag preparation; the course of these nerves has in fact been frequently described — they do not present such extraordinary aberrations. Unless we take refuge in unbridled speculation and surmise without real histological evidence that the cardiac nerves form a continuous net-work, there is little escape from the conclusion that the conduction in these experiments is not by any means of nerves. It is therefore highly probable that *the synchronism of the mammalian ventricles is maintained through muscular and not through nervous connections.*

Having come thus far it is necessary to reflect on the part the auricle may play in the coördination of the ventricular contractions. The auricles are connected with the ventricles by both muscular and nervous tissue. It is through the auricle that the excitation wave reaches the ventricle, and through the auricle also pass impulses that modify the character of the ventricular contraction. What more probable than that the auricles, or the sinus venosus operating through the auricle, should have a hand in the coördination of the ventricles? If the right ventricle be separated entirely from the left but the connections of both ventricles with the auricle allowed to remain undisturbed, will not the synchronism of the ventricles be preserved?

The first experiment in this series was made November 17, 1898. The experiments began with an attempt to coördinate portions of one ventricle through their connection with the auricles. Typical protocols are as follows.

Experiment November 23, 1898. A dog was bled in the manner already described, the heart excised, and a cannula tied into the circumflex branch of the left coronary artery. The part supplied by this artery, embracing nearly the whole of the left ventricle, was now separated from the remainder of the ventricles by incisions made near the anterior and posterior margins of the interventricular septum respectively. Care was taken not to injure the auricle. On supplying the separated part with blood through the nutrient artery, it began to beat in the usual manner. The auricle also contracted, but the contractions were feeble, and were not always followed by contractions of the ventricle. The ventricle was now divided into several strips by incisions running from the apex nearly to the auricle, between the principal branches of the ramus circumflexus, so that each strip continued to receive blood from a branch of the artery. All the strips continued to beat synchronously. One of the strips was now entirely separated from the rest of the ventricle by continuing the incisions which bounded the strip on its two sides through the auriculo-ventricular groove. The circumflex artery was carefully avoided, so that the nutrition of the strip now isolated from the ventricles was preserved. The broad connection of the strip with the auricle was left untouched. Upon the last muscular connection with the ventricle being severed, the rate of beat of the isolated part suddenly changed. It no longer contracted synchronously with the rest of the ventricle but at a slower rate.

Similar experiments were repeated many times in the three dogs and ten cats which served for this portion of the investigation. The result has invariably been the same. Any part of either ventricle can be separated almost entirely from the contiguous portions without interfering with the synchronism of the whole; the division can be completed on one side by an incision crossing the auriculo-ventricular groove without disturbing the synchronism; the remaining side can be reduced to a very small muscular bridge, and the parts will still beat in unison; but so soon as the bridge is destroyed, the synchronism is destroyed, although both portions of the ventricle possess their normal connections with the auricle. Very rarely — only once in my numerous experiments — a few synchronous beats in the two portions of ventricle may indeed be witnessed, but the observer will readily perceive that these are chance coincidences of two separate and variable rhythms. Experiments of this sort therefore give a negative reply to the question whether the ventricles can be coördinated through the auricles.

The procedure just described is open to the objection that the auricle and ventricle as a rule did not beat in their normal sequence in these experiments, indicating perhaps that the conduction across the auriculo-ventricular groove was more or less at fault. Thus the failure of the auricle to coördinate the ventricles might possibly be due to a disturbance of conduction between auricle and ventricle.[1] The method should maintain the normal sequence of contraction. Only when the isolation of a portion of the ventricle does not disturb the normal sequence between the auricles and the remainder of the ventricle can we be sure that the independent beat of the separated part is not caused by a general failure in conduction from auricle to ventricle. The objection is valid; we cannot be sure; though the probability of error, considering the number of observations, is not great. The method should be improved.

The necessary improvement in the method was secured in the following way.

Experiment December 8, 1898. A cat anæsthetized with ether was bled from the left carotid artery, but not to excess, and the blood added to that collected from two other cats. The animal was now tracheotomized, artificial respiration begun, the chest opened, the left carotid ligated, and a cannula tied into the innominate artery. The right coronary artery was laid bare near its origin. The cat was then placed on the inclined support described in Miss Hyde's paper in this Journal,[1] and the cannula connected with the reservoir containing defibrinated blood[2] at a temperature of about 33°C. and a pressure of about 80 mm. Hg. The aorta was then ligated just distal to the origin of the left carotid artery, and the right ventricle opened near the apex. On opening the stop-cock between the feeding bottle and the innominate artery, the blood passed from the aorta through the coronary vessels to the right heart and thence into the chest cavity, gradually filling the deep pocket formed by the diaphragm and the lower part of the thorax. From this reservoir it was removed from time to time, filtered, and replaced in the feeding bottle. The action of the heart was thoroughly satisfactory; all parts contracted vigorously and each contraction of the auricles was followed by a contraction of the ventricles.

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[1] The difficulty here mentioned is not due to a reduction of vitality by the excessive bleeding of the animal before the experiment; in all the later experiments the greater part of the blood was obtained from one animal and the heart to be perfused was taken from a fresh animal, from which very little blood was drawn.

The right ventricle was now slit up, parallel with the anterior interventricular furrow, the section passing beneath the exposed right coronary artery completely across the auriculo-ventricular groove. The synchronism of the ventricles and the normal auriculo-ventricular sequence was not altered by this. A second incision was made near to and parallel with the posterior interventricular furrow almost to the auricle. All parts continued to beat as before. The section was extended across the bridge of ventricular substance and the auriculo-ventricular groove. The right ventricle, thus wholly isolated from the left, immediately began to beat with a slower rhythm. The remainder of the heart continued at the old rate, each auricular contraction being followed by a ventricular contraction.

Obviously in this experiment there can be no question of the preservation of the normal power of conduction; the ample natural connection of the isolated portion of the right ventricle with the auricles was left untouched; the remainder of the ventricles beat in sequence with the auricles; it cannot be supposed that conduction to the isolated portion was instantaneously lost in consequence of the mechanical injury of an operation repeatedly shown not to entail such consequences; the sections made in this and previous experiments never interfered with coördination or sequence unless they completed the separation of one portion of the ventricle from the rest of the ventricular mass; it may be mentioned, also, that had the effects observed been due to mechanical injury, they should have shown themselves on both sides of the incision instead of only on the side separated; the cause of the alteration in the separated portion cannot, then, be mechanical injury. The experiments demonstrate that any portion of the ventricle will beat synchronously[1] with any other portion, so long as the two are connected by muscle tissue, but that synchronism immediately fails when the muscle bridge is broken, in spite of the fact that both portions may retain their normal connection with the uninjured auricles. Further than this it would be unsafe to venture. The facts in our possession do not permit a positive statement as to the nature of the coördination impulse, and it is undesirable to add another speculation to the mass with which the physiology of the heart is cumbered.

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[1] Miss I. H. Hyde: This journal, 1898, i, p. 215.

[2] The blood contained some 0.8 per cent sodium chloride solution, but it may be remarked once more that undiluted blood will serve, though less convenient.

On the same day two other instructive experiments of this kind were performed. In the first of these the isolated portion of the right ventricle beat with the same frequency as the remainder of the heart, but with a different rhythm; the beats were not synchronous with the other portion of the ventricles. In the last of the three experiments, the isolated portion of the right ventricle continued to beat as if nothing had happened; the synchronism of the two portions of the ventricles was unchanged. The exception, however, proved the rule, for post mortem it was found that the first incision — that made along the left anterior border of the right ventricle — had not entirely severed the tissues near the auriculo-ventricular groove; a few muscular fibres still connected the apparently isolated portion with the rest of the ventricle.

It is true that the result here recorded is in some degree a negative one. Yet the number and character of the experiments seem to leave no room for doubt, and I do not therefore hesitate to conclude that *the synchronism of the ventricular contractions is not a function of the auricles but is managed by the ventricles themselves.*

SUMMARY.

1. The synchronism of the mammalian ventricles is not dependent on nerve cells.
2. The synchronism of the mammalian ventricles is probably maintained through muscular and not through nervous connections.
3. The synchronism of the ventricular contractions is not a function of the auricles but is managed by the ventricles themselves.

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[1] It is of course understood that time is required for the excitation to pass from one part of the ventricle to another, — the synchronism is relative, not absolute.

ON THE PATHS OF ABSORPTION FOR PROTEIDS.

By LAFAYETTE B. MENDEL.

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THE text-books of physiology agree, at present, in assigning to the portal circulation the task of transporting away from the alimentary canal ingested proteids after they have been modified by the natural digestive processes. The experimental evidence in favor of this view is familiar, and has scarcely been questioned; recently, however, Asher and Barbèra[1] have published the results of an experiment which leads them to conclude that the thoracic duct forms — though perhaps only to a small extent — a channel for the transportation of digested proteids to the blood. These investigators experimented upon a large dog having a well-healed gastric fistula. A cannula was introduced into the thoracic duct of the animal after it had fasted for sixty hours. During continued narcosis the hunger-lymph was collected for an hour; two hundred grams of dry albumin (from blood) were then introduced into the stomach through the fistula and thereupon the lymph collected for six hours in hourly portions. Total solids, ash, and total nitrogen were determined in the seven portions, and the proteid content of the lymph was calculated from these data. The results obtained may be summarized as follows:

Experiment of Asher and Barbèra.

AVERAGE COMPOSITION OF LYMPH, PER HOUR.

		Before Proteid feeding.	*After Proteid feeding.*
Lymph,	grams	16.50	27.91
Total Solids,	grams	1.15	2.18
" "	per cent	7.05	7.81
Ash,	grams	0.08	0.10
"	per cent	0.49	0.35
Nitrogen,	grams	0.132	0.251
"	per cent	0.809	0.899
Proteid (N × 6.25),	grams	0.825	1.572
"	per cent	5.056	5.632

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[1] ASHER and BARBÈRA: Centralblatt f. Physiologie, 1897, **xi**, p. 403. "Der Brustgang betheiligt sich also nach Eiweissnahrung an der Fortführung des Eiweisses in das Blut, wenn auch in geringer Menge." (*Ibid.*, pp. 406–7.)

In connection with the data obtained, Asher and Barbèra point out that the curves plotted to indicate the changes in the composition of the lymph from hour to hour show a striking similarity to those obtained for the hourly nitrogen excretion in the urine after meals;[1] and the authors are inclined to attach significance to the correspondence between the curves.

In support of earlier experiments[2] demonstrating that — in man — the absorption of ingested proteids is not ordinarily a function of the lymphatics, I. Munk[3] has published a brief review of the experiment of Asher and Barbèra. He points out that the total proteid found in the lymph during the six hours of the absorption period exceeds the proteid in the hunger-lymph, calculated for an equal period, by only 4.485 grams, *i. e.*,

Total proteid in "absorption" lymph = 9 435 grams
" " " "hunger" " (6 × 0.825) = 4.95 "
Proteid presumably absorbed into lymphatics = 4.485 "

Of the two hundred grams of proteid introduced into the dog's stomach, one hundred and thirty grams were recovered at the end of the experiment. Accordingly, since seventy grams of the albumin introduced had disappeared from the alimentary tract, the additional quantity of proteid found in the lymph during six hours of absorption corresponds to only 6.4 per cent of the proteid absorbed. In other words, 93.6 per cent of the material absorbed presumably passed into the circulation by channels other than those leading to the thoracic duct. Munk further points out that these results were obtained with excessive quantities of proteid: two hundred grams being given to the dog at one time.

I have repeated the experiment of Asher and Barbèra with modifications in two respects. First, a smaller quantity of proteid was fed; and secondly, the proteid employed was a readily soluble one — Witte's "pepton" — which previous experience had shown to be satisfactorily utilized by dogs.[4] A gastric fistula had been made on the 14-kilo animal several months previously. The wound was perfectly healed and the dog in good health.[1] After forty-eight hours fasting a glass cannula was tied into the thoracic duct during morphine narcosis (0.16 gram morphine sulphate + 0.016 gram atropine sulphate subcutaneously), a little chloroform-ether being administered when necessary. The dog remained in narcosis during the experiment. The portion of lymph collected during the first ten minutes after introduction of the cannula was not retained, since owing to the operative procedure the flow of lymph is at first always somewhat more rapid than subsequently.[2] The "hunger" lymph was collected during an hour; thereupon fifty-five grams of Witte's "pepton" containing 14.0 per cent nitrogen were introduced with 125 cb.cm. water through the fistula into the stomach. The lymph was then collected in hourly portions during the six succeeding hours. The animal was killed by bleeding and the stomach contents removed and examined. The intestine was found empty; the appearance of the gastric and intestinal mucosa was normal. The stomach contained 205 cb.cm. fluid with mucous flocks in suspension. The filtered gastric contents, composed largely of primary proteoses with a little true peptone, furnished the following data on analysis:

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[1] Cf. TSCHLENOFF: Correspondenzblatt f. Schweitzer Aerzte, 1896, p. 1; VERAGUTH: Journal of physiology, 1897, xxi, p. 112; HOPKINS and HOPE: *ibid.*, 1898, xxiii, p. 271; HOPKINS: Schaefer's Text-book of physiology, 1898, i, p. 585.

[2] MUNK and ROSENSTEIN: Virchow's Arch. f. d. exper. Pathologie, 1891, cxxiii, p. 496.

[3] I. MUNK: Centralblatt für Physiologie, 1897, xi, p. 585.

[4] Cf. MENDEL and JACKSON: This journal, 1898, ii, pp. 10–11.

Total solids, ...	30 23	grams
Total nitrogen (Kjeldahl), ...	4.42	"
Nitrogen in total solids, ...	14.6	per cent
Total acidity (equivalent to HCl), ...	0.77	"

No *free* HCl present.

The lymph portions were analyzed in the customary manner, nitrogen being determined by the Kjeldahl method and the proteid calculated from the figures thus obtained. The results are summarized in the accompanying table.

The fifty-five grams of Witte's "pepton" introduced into the stomach contained 7.70 grams of nitrogen. At the end of the experiment unabsorbed proteid containing 4.42 grams nitrogen was recovered from the stomach. The nitrogen in the "pepton" absorbed thus amounted to 7.70 — 4.42 = 3.28 grams, equivalent to 23.4 grams of Witte's "pepton." Nearly one half the proteid fed had thus been absorbed; in Asher and Barbèra's experiment about one third of the albumin disappeared. In my experiment the total nitrogen in the

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[1] The form of gastric cannula used has been described in This journal, 1898, i, p. 191.

[2] Cf. HEIDENHAIN: Arch. f. d. ges. Physiol., 1891, xlix, p. 215.

TABLE OF ANALYSES OF LYMPH.

Time.	Amount Collected.	Total Solids.		Ash.		Nitrogen.		Proteid (N. × 6.25).	
hours	grams	grams	per cent	grams	per cent	grams	per cent	grams	per cent
1. (Before feeding)	13.46	0.551	4.09	0.122	0.90	0.0609	0.45	0.381	2.83
2. (After feeding)	16.59	.625	3.83	.147	.88	.0674	.40	.421	2.53
3. " "	11.50	.472	4.10	.104	.90	.0475	.41	.297	2.58
4. " "	12.64	.548	4.33	.105	.83	.0614	.48	.384	3.03
5. " "	8.79	.393	4.47	.068	.77	.0454	.51	.284	3.23
6. " "	14.60	.674	4.61	.125	.85	.0768	.52	.480	3.28
7. " "	15.90	.708	4.45	.128	.80	.0757	.47	.473	2.97
Six fasting hrs. (calculated) .	80.76	3.306	4.09	0.732	0.90	0.3654	0.45	2.286	2.83
Six absorption hrs. (2.—7.) . . .	80.02	3.420	4.27	.677	.84	.3742	.46	2.339	2.92

lymph during the absorption period amounted to 0.374 gram; the nitrogen-content of the starvation lymph (1.), calculated for an equal period, would amount to 6 × 0.0609 = 0.365 gram. The difference between these figures — 0.0088 gram N. = 0.053 gram proteid — which may be attributed to the proteid absorbed, is insignificantly small in amount, and is far smaller than the corresponding figure obtained by Asher and Barbèra with a considerably larger quantity of proteid. Neither the total quantity of lymph collected nor the percentage of proteid present was increased during the six hours of absorption.

With reference to a possible correspondence between lymph flow and urea excretion after proteid absorption it may be remarked that — according to Tschlenoff — if peptone is ingested instead of ordinary proteids, the maximum urea excretion is reached in the second hour. To emphasize the comparison — as Asher and Barbèra have done — between the lymph flow and the urea excretion observed in quite different experiments seems to me of doubtful value; for the lymph flow is readily influenced by purely mechanical factors. Thus it is quite possible that in my experiment movements of defecation observed in the dog in the 6th hour may explain the increased flow of lymph unaccompanied by any alteration in the percentage of proteids; just as slight pressure on the abdomen will immediately cause an increase in the flow.

Asher and Barbèra[1] have formulated the theory that the lymph is a product of metabolism in the glands of the body, and they have attempted to demonstrate that the lymph flow increases in all cases where there is accelerated glandular activity. In view of the work accomplished by the alimentary epithelium and the liver during the digestion and absorption of proteids, the new theory demands that an increased flow of lymph should result after proteid feeding. The present experiment with "pepton" failed to yield results in conformity with this view; it might, however, be said by the defenders of the theory that no pronounced increase in lymph flow is to be expected during the absorption of soluble and readily diffusible products like Witte's "pepton," since the work of the intestines is comparatively slight under these conditions. Such, at least, is the explanation which they have applied to the absence of accelerated lymph flow in carbohydrate nutrition.

The results of the absorption experiment with moderate quantities of a soluble proteid fail to modify the current statements regarding the paths by which proteids are absorbed; *i. e.*, under ordinary circumstances by far the greater share in the process must still be delegated to the capillaries of the villi.

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[1] ASHER and BARBÈRA: Zeitschr. f. Biologie, 1898, xxxvi, p. 210; ASHER: IV International Physiological Congress; Centralbl. f. Physiol., 1898, xii, p. 486.

A CHEMICO-PHYSIOLOGICAL STUDY OF CERTAIN DERIVATIVES OF THE PROTEIDS.

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THE marked activity which has characterized the study of the proteids and their primary digestion or cleavage products during the last decade has been especially manifest in two distinct directions. In the first place, effort has been directed toward a study of the various transitions which these substances undergo in the several phases of digestion and nutrition, with a view to obtaining clearer insight into the genetic relationship of the various chemical products and incidentally into their chemical nature. In the second place, much attention has been given to a study of the physiological action of the more characteristic products of gastric and pancreatic proteolysis, and more especially the behavior of these products when introduced into the system through channels other than the gastro-intestinal tract. This latter phase of physiological research has been carried out in the majority of cases with a mixed product resulting from the digestion of blood-fibrin with gastric juice, known as " Witte's

Pepton," and composed of a variable proportion of proteoses with some true peptone.

It is needless to refer here in detail to the extensive literature which these latter investigations have created. It will suffice to recall the fact that the more important observations on the effect of so-called "peptones" when introduced directly into the blood current were made by Schmidt-Mülheim[1] and Fano[2] in Ludwig's laboratory. The results they obtained have in part become classic, and supplemented by certain later observations made by other investigators may be briefly summarized as follows: The primary products of digestion (proteoses) when introduced directly into the circulation of dogs in doses from three to five decigrams per kilogram of body-weight produce a rapid and marked fall of arterial blood pressure, which slowly returns to the normal. If after the injection of the proteoses blood be withdrawn from an artery, it fails to clot as rapidly as under normal conditions, or may even remain liquid indefinitely. The intensity of the effects produced depends in part on the rapidity with which the substance is introduced into the circulation and to a lesser degree on the quantity of material employed. Further, when the effects of an injection have disappeared a second similar injection may fail to produce the characteristic action, a certain degree of immunity having been established. Coincident with these results there usually occurs a very marked increase in the flow of lymph in the thoracic duct, together with a decreased coagulability of this fluid. Other changes in the urinary secretion and respiration are less constant, and have been less thoroughly investigated.

These statements will, we believe, receive general acceptance as expressing well-established truths. There remain, however, a large number of problems to the solution of some of which the experimental work of this paper is directed. Thus, the questions arise: Are the various physiological results — fall of arterial pressure, loss of blood coagulability, lymphagogic influence, etc. — genetically related or independent phenomena? Is the influence exercised directly upon the blood or indirectly through specific organs or tissues? Are all organisms equally susceptible to these actions? Are the effects produced common to all decomposition or cleavage products of proteids? What light do the phenomena throw upon normal or abnormal processes in the body?

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[1] SCHMIDT-MÜLHEIM: Archiv f. Physiol., 1880, p. 30.

[2] FANO: *Ibid.*, 1881, p. 277.

Such are some of the problems presented. Recent work by a number of investigators, however, has thrown light in many directions and revealed a multitude of facts which promise advance in this particular field. Still, few investigators have attempted to use for study more characteristic or more definite chemical products than the earlier workers had at their command, or where this has been attempted there has in some cases at least existed a certain degree of indefiniteness concerning the exact nature of the substances employed. This is the more to be regretted as there are many facts which tend to show that the primary cleavage products of the proteids may differ from each other in their chemical constitution, and if so one might naturally look for pronounced variations in physiological behavior. Further, it unfortunately happens that previous experimental data obtained with isolated proteoses and peptones are frequently deficient or entirely wanting on many points of interest. We therefore present our results obtained with various types of proteid cleavage products in the hope that they may contribute somewhat to a broader and more concise knowledge of the subject. The several products we have made use of are fully described as regards mode of preparation, composition, and chemical characters in another section of this paper. Their physiological action is described in the following pages.

THE PHYSIOLOGICAL EFFECTS OF SOME CLEAVAGE PRODUCTS OF THE PROTEIDS WHEN INTRODUCED DIRECTLY INTO THE CIRCULATION.

Technique. — Our experiments were carried out exclusively on dogs. The animals were made to fast for at least twenty-four hours previous to the operative procedure; they were narcotized by a subcutaneous injection of morphine mixed with atropine, and a mixture of chloroform and ether was administered during the operative interference and at other times when necessary. Arterial pressure was recorded by a mercurial manometer connected with the carotid, or femoral artery, and the injections were made into one of the facial veins from a burette attached to a glass cannula. Intervals of one second were recorded beside the blood pressure curve by means of a metronome time-marker. In observing the rate at which the blood coägulated, portions were drawn from the right femoral artery. The cannula was removed after each withdrawal of blood and thoroughly cleansed, and the blood was allowed to escape for a second or two before the next portion was taken. The samples of 2 c.c. each were received in graduated cylinders with a calibre of one centimetre. The "rate of coagulation" was taken to be the time which elapsed before the cylinder could be inverted without the loss of a drop.

The elimination of the substances through the kidneys, as well as the rate of flow of the urine, was studied by collecting the urine from cannulæ introduced directly into the ureters. In those experiments in which the lymph was collected and analyzed, the directions of Heidenhain[1] were followed.

The proteoses, peptones, etc., studied were usually dissolved in 0.7 per cent sodium chloride solution. With substances insoluble in dilute saline fluids, such as heteroalbumose and antialbumid, it was found best to use a few drops of ammonium hydroxide to facilitate solution, any excess of ammonia being driven off by heat. The volume of fluid injected was ordinarily 4 c.c. per kilo of body-weight up to a maximum of 50 c.c. The doses of the different substances employed were varied in order to ascertain their relative activity. The injections were accomplished as rapidly as possible, and seldom occupied more than thirty seconds. Indeed, many of the tracings show a slight initial rise of blood pressure due probably to the increased volume of the blood reaching the heart, before the fall caused by the substances injected.

Specific substances studied. — In selecting substances for study we have been influenced by the fact that the evidence already at hand is sufficient for establishing the general action of mixed proteoses and peptones. It has therefore seemed to us desirable to study especially the physiological action of certain well-defined cleavage products with a view to obtaining evidence of the action of the individual bodies present in the ordinary digestion products of the proteids. Thus, an investigation of the physiological action of the immediate precursors of antipeptone has seemed especially desirable in view of the discordant observations of previous investigators with this product and also in view of the peculiar significance which is attached to this substance through recent chemico-physiological research.[2] Furthermore, we have been impressed with the importance of having true antipeptone for experiment, and consequently, we have employed pure antialbumid as the mother substance from which to prepare the antipeptone. Following is a list of the substances studied, all of which were prepared with great care according to the methods detailed in the second part of this paper.

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[1] HEIDENHAIN: Archiv f. d. ges. Physiol., 1891, xlix, p. 209.

[2] SIEGFRIED et al.

Antialbumid (A), formed from coagulated egg-albumin by the action of dilute sulphuric acid at 100° C., and purified by digestion with pepsin-acid, etc.
Antialbumoses (B), formed by the digestion of the above antialbumid with gastric juice.
Antialbumoses (C), formed by the digestion of the above antialbumid with pancreatic juice.
Antipeptone (D), formed by the digestion of antialbumid with pancreatic juice.
Antialbumid (E), a more insoluble form, separating from an alkaline solution of the above antialbumid when subjected to pancreatic digestion.
Antialbumid (F), prepared from crystallized edestin by the action of dilute sulphuric acid.
Hemialbumoses (G), formed from coagulated egg-albumin by dilute sulphuric acid.
Heteroalbumose (H), formed in a similar manner.
Hemipeptone (I), formed by the same method as the hemialbumoses.
Protogelatose (J), formed from pure gelatin by gastric digestion.
Deuterogelatose (K), formed by pancreatic digestion.
Gelatin-peptone (L), formed by pancreatic digestion.
Albumose-like body (M), formed from coagulated egg-albumin by the action of dilute sulphuric acid at 100° C.

Influence on blood pressure. — The first more comprehensive and systematic study of the physiological action of proteoses and peptone individually was made by Grosjean.[1] This investigator prepared the several products from blood-fibrin by artificial gastric digestion, using the methods of Kühne and Chittenden in isolating and purifying the mixed proteoses and peptone. Two products only were prepared: *i. e.*, "propeptone," a varying mixture of primary and secondary proteoses, and "peptone" or amphopeptone. These products were introduced intravenously into the circulation of dogs and other animals, and arterial blood pressure registered. Grosjean concluded from his experiments that "propeptone" introduced into the circulation causes a considerable fall of arterial pressure, the depression occurring at once after the injection and quickly reaching its maximum. Further, the extent to which arterial pressure is lowered is apparently not influenced by the size of the dose, except when the latter amounts to less than 15 centigrams per kilo of body-weight, in which case the depression is much less marked. Moreover, as a rule, the pressure once lowered is very slow in returning to the normal. Thus, in one experiment where 1 gram of "propeptone" per kilo was injected, arterial pressure was still below the normal three and a half hours after the injection. With peptone, there is likewise produced a fall of arterial pressure which, however, is less marked, slower in reaching its maximum, and of shorter duration than that caused by like doses of "propeptone." Further, when the pressure returns toward the normal it frequently rises slightly above the original height, after which it again falls, although this second depression is slight as compared with the first.

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[1] GROSJEAN: Archives de biologie, 1892, xii. This paper contains references to the earlier literature.

Arthus and Huber[1] working with gelatoses and caseoses formed by pancreatic digestion, observed that when these bodies were introduced intravenously into dogs in large doses (2 grams gelatoses per kilo; 1.5 grams caseoses per kilo), and the injections carried out rapidly, there was a marked lowering of blood pressure comparable to that obtained with the fibrin proteoses. Here, too, no attempt was made to differentiate the mixed caseoses or gelatoses into more specific products.

In a recent paper from this laboratory[2] attention was called to the action of deuteroalbumose and peptone obtained by the proteolysis of egg-albumin with papaïn. The experiments showed an immediate lowering of blood pressure when these products, in doses of 0.5 gram per kilo of body-weight, were injected into the circulation, the pressure, however, soon returning to the normal.

Thompson[3] has investigated the specific action of Witte's "pepton" on the vasomotor system, and more recently this study has been extended[4] to include the action of pure peptone, antipeptone, and deuteroalbumose, although the details of these latter experiments have not yet been published. The results obtained agree in a general way with those already recorded by Grosjean. They indicate, however, that smaller doses of these products than has generally been supposed, suffice to produce physiological effects. It is furthermore definitely shown that lowering of arterial pressure is due to a vascular dilatation as the result of a peripheral or local action on the blood vessels. No central nervous influences could be demonstrated. This vasomotor action is not confined to the splanchnic area, but is common to all the blood vessels, and is exercised presumably upon the endings of the vasomotor nerves and on the muscles of the blood vessels, — the decreased irritability of the neuro-muscular apparatus making the latter incapable of responding to the normal vaso-constrictor impulses. In addition, it is to be noted that Thompson observed that deuteroproteose produced a far more profound and enduring influence on arterial pressure than pure peptone, but less so than the same dose of Witte's "pepton." Hence, deuteroproteose cannot be regarded as the most potent constituent of Witte's "pepton;" a statement which agrees with Pollitzer's[1] observations. Lastly, in agreement with other workers (Pollitzer, Spiro, and Ellinger[2]) Thompson found that antipeptone contrasts very noticeably with the other products of proteid digestion studied in its action on blood pressure. The fall of pressure produced was *temporary* at most.

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[1] ARTHUS and HUBER: Archives de physiologie, 1896, p. 857.

[2] CHITTENDEN, MENDEL, and MCDERMOTT: This journal, 1898, i, p. 255.

[3] THOMPSON: Journal of physiology, 1896, xx, p. 455.

[4] THOMPSON: Interim report of a committee consisting of Professors Schäfer, Sherrington, Boyce, and Thompson. Report by the Secretary of the British Association for the Advancement of Science, 1896–97.

Our experiments on blood pressure, the results of which are summarized in the following table, indicate that antialbumid, a product whose physiological action has not heretofore been investigated, agrees with the antipeptone derived from it in producing comparatively slight effects on mean arterial pressure. In like doses, however, antialbumid is somewhat more vigorous in action than antipeptone. Further, it is evident that antipeptone derived from antialbumid produces essentially the same results as antipeptone formed by the direct action of pancreatic juice on a natural proteid. Antialbumoses, on the other hand, act most energetically in lowering blood pressure. In no case was a return of mean arterial pressure to the normal observed within an hour after injection of the various albumoses studied. The same was true of the hemialbumoses. The albumoses of different origin act qualitatively and quantitatively alike in great degree, heteroalbumose being apparently most vigorous in its effects. This corresponds with the observations of Pollitzer,[3] and we are inclined to attribute the pronounced effects produced by Witte's "pepton" to the considerable admixture of heteroproteose, which it has repeatedly been shown to contain.[4]

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[1] POLLITZER: Journal of physiology, 1886, vii, p. 283.

[2] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

[3] POLLITZER: *loc. cit.*

[4] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1884, xx, p. 15.

TABLE, SHOWING EFFECTS UPON ARTERIAL PRESSURE, MEASURED IN MILLIMETRES OF MERCURY.

Experiment.	Weight of animal; kilos.	Grams per kilo injected.	Nature of the substance employed.	Pressure before injection.	Pressure just after injection.	5 minutes after.	15 minutes after.	30 minutes after.	1 hour after.	1½ hours after.	2 hours after.
I	8.8	0.3	Antialbumoses (B) .	180	90	40	45	50		75	
II	6.2	0.3	“ “ .	85	45	30	35	50	70		
III	11.3	0.3	Antialbumoses (C) .	140	30	30	30	45	55	95	110
IV	6.0	0.3	“ “ .	130	35		105	125			
V	18.0	0.3	Hemialbumoses (G) .	170	65	40	38	50	65	85	
VI	7.5	0.18	Heteroalbumose (H) .	160	135	30	20	30	45	45	45
VII	9.8	0.2	“ “ .	115	75	30		75			
VIII	7.0	0.3	Antialbumid[1] (A) . .	80	20	45	90	90			
IX	6.0	0.3	“ “ . .	150	80	40	115	125			
X	5.4	0.3	“ “ . .	130	70	125	150	145	150	135	
XI	7.6	0.14	“ “ . .	150	55	40	115	125	140	150	
XII	14.0	0.15	“ “ . .	140	90	135	130				
XIII	30.0	0.03	“ “ . .	65	30	20	45	60	65		
XIV	8.4	0.17	“ “ . .	140	140	140	140	140			
XV	6.8	0.07	Antialbumid (F) . .	95	95	85	110	100			
XVI	9.0	0.3	Antipeptone (D) . .	170	160	160	190	170			
XVII	6.5	0.3	“ “ . .	90	40	80	90	90			
XVIII	9.0	0.3	“ “ . .	110	60	135	145	150			
XXXI	4.5	0.45	“ “ . .	120	55	70	140	140			
XIX	11.2	0.3	Hemipeptone (I) . .	125	20	120	105			80	
XX	6.5	0.3	“ “ . .	125	60	110	130	130	130		
XXI	13.5	0.5	“ “ . .	130	40	85	130	120	145	145	
XXII	6.4	1.0	“ “ . .	115	50	20	45	60	110		
XXIII	5.0	0.3	Albumose-like body (M)	110	40	20	50	110	110		
XXIV	5.0	1.5	Protogelatose (J) . .	140	55	135	150	135			
XXV	4.2	0.3	Gelatin-peptone (L) .	100	75	100	95	100	100		
XXVI	6.0	0.6	“ “ .	105	40	60	115	120	115		
XXVII	21.0	0.35	Deuterogelatose (K) .	115	115	115	120	120	120		
XXVIII	7.0	0.5	“ “ .	120	50	95	115	120	120		
XXIX	15.0	0.7	“ “ .	130	60	120	150	150			
XXX	6.5	1.0	“ “ .	135	35	65	125	140	120		

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[1] All of the samples of antialbumid were dissolved by the aid of a little dilute ammonia except in Experiment XIV, where dilute sodium carbonate was used.

Our experiments with hemipeptone agree with the results obtained by Grosjean and later by Thompson with amphopeptone isolated by Kühne's method. Further, between hemipeptone and antipeptone there is little or no difference of action when like amounts of substance are employed. It is interesting, however, to contrast the action of equal doses of hemipeptone (XIX–XX) with that of the hemialbumoses (V) from the same source. It is only with doses of 1.0 gram of hemipeptone per kilo that a prolonged fall of pressure is obtainable, and even with this quantity the pressure again resumes its normal within an hour. These facts seemingly suggest more or less pronounced difference in the chemical structure of the proteose and peptone molecules, — a point which we have taken occasion to emphasize in other connections.[1]

The experiments with protogelatose and deuterogelatose in agreement with the observations of Arthus and Huber, fail to show any such marked influence as is obtained with the corresponding albumoses. True gelatin-peptone, on the other hand, approaches hemipeptone and antipeptone in the intensity of its action. The great difference which the albuminoid products show, quantitatively at least, in contrast with the derivatives of the native proteids serves to emphasize the other striking differences between gelatin and true proteids as regards physiological equivalence.[2]

Lastly, attention is directed to the slight initial rise in pressure immediately succeeding the injection of the various substances into the circulation as shown in the tracings. This rise, which is undoubtedly due to the rapid entrance of the fluid into the vascular system,[3] gives evidence of the rapidity with which the injections were carried out. This fact is not without significance, since the variation in effects of intravenous injections of proteoses has been demonstrated (cf. Thompson, p. 119) to be dependent in part on the rapidity of the injections. It seems reasonable therefore to assume that the intensity of vasomotor effects is not so much a function directly of the absolute quantity injected as of the quantity in the circulation per unit of time, or in other words, of the concentration of the substance in the blood.

The accompanying blood pressure tracings have been selected from typical experiments. They were recorded with a mercurial manometer, as already described. The time is recorded in seconds, and the line of zero pressure is likewise recorded. The curves all read from right to left, and only such portions have been reproduced here as furnish evidence of the observations discussed in the preceding pages.

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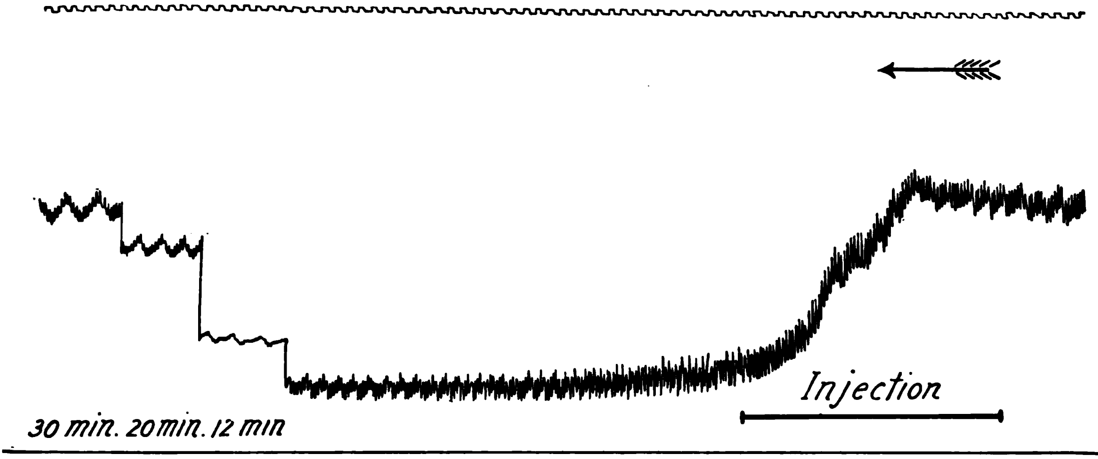
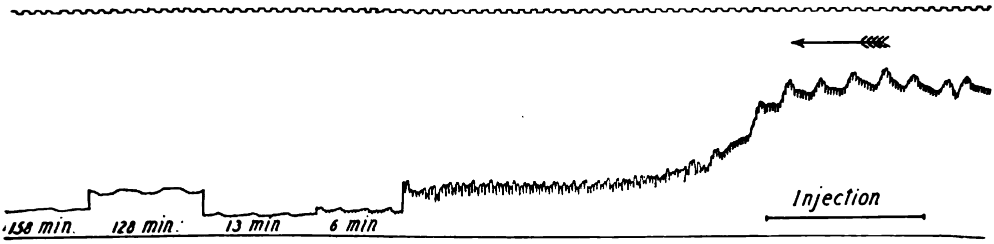
[1] CHITTENDEN and MENDEL: Journal of physiology, 1894, xvii, p. 78. CHITTENDEN: Digestive proteolysis, 1894, p. 42. Also recent papers from Hofmeister's laboratory. Zeitschr. f. physiol. Chem., 1897–98.

[2] Cf. I. MUNK: Archiv f. d. ges. Physiol., 1894, lviii, p. 309. Also MENDEL and JACKSON: This journal, 1898, vol. ii, p. 21.

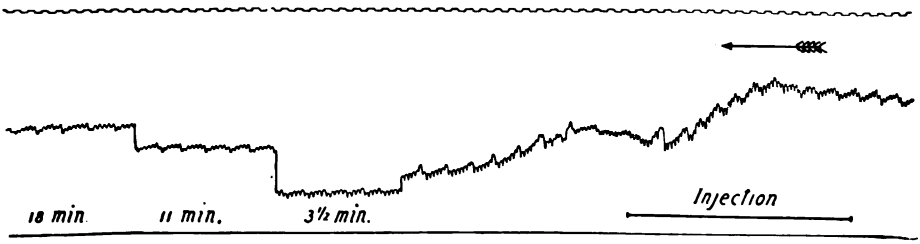
[3] Cf. THOMPSON: Journal of physiology, 1896, xx, p. 455.



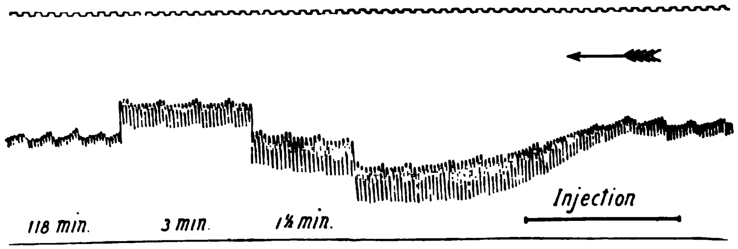
Experiment IV. 6 kilo dog. Injection of ANTIALBUMOSES (C). 0.3 gram per kilo. One half the original size.



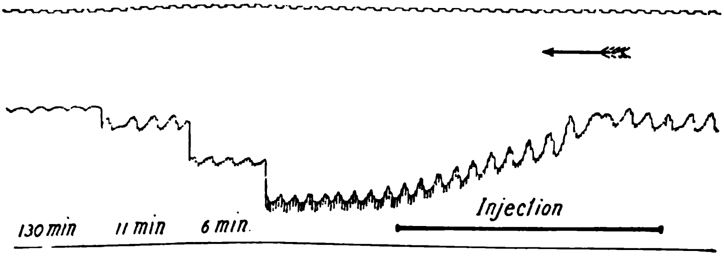
Experiment VI. 7.5 kilo dog. Injection of HETEROALBUMOSE (H). 0.18 gram per kilo. (Cf. tracing Exper. *XXIX b.*) One fourth the original size.



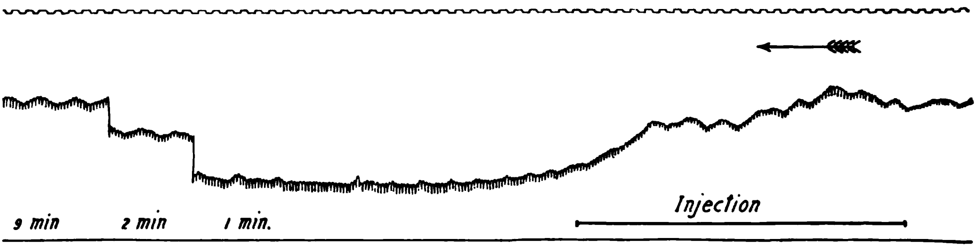
Experiment IX. 6 kilo dog. Injection of ANTIALBUMID (A). 0.2 gram per kilo. One fourth the original size.



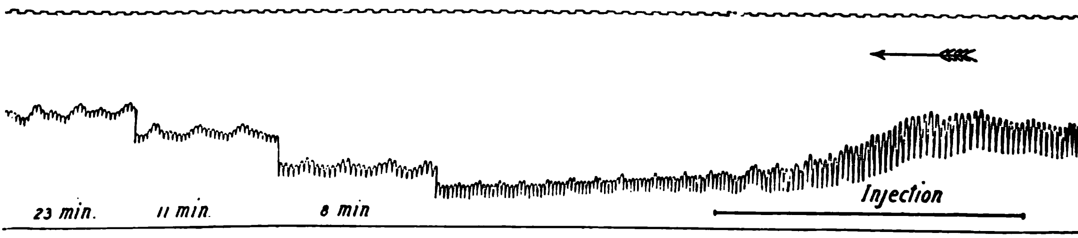
Experiment XVIII. 9 kilo dog. Injection of ANTIPEPTONE (D). 0.3 gram per kilo. One fourth the original size.



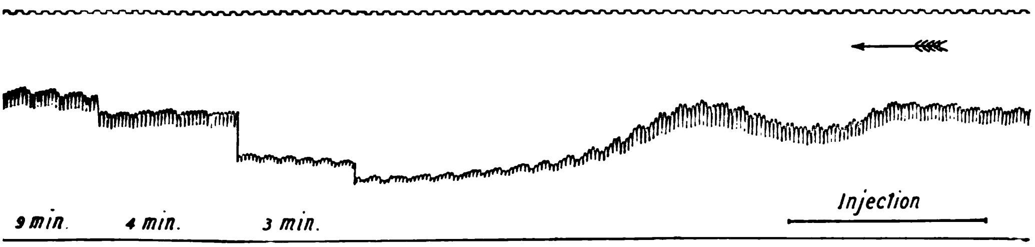
Experiment XXI. 13.5 kilo dog. Cannula in thoracic duct. Injection of HEMIPEPTONE (I). 0.5 gram per kilo. One fourth the original size.



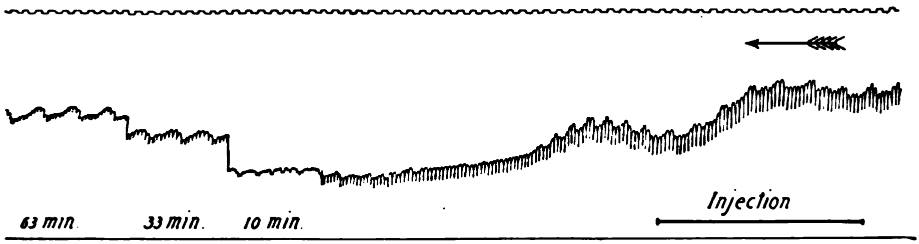
Experiment XXIV. 5 kilo dog. Injection of PROTOGELATOSE (J). 1.5 grams per kilo. One fourth the original size.



Experiment XXVI. 6 kilo dog. Injection of GELATINPEPTONE (L). 0.6 gram per kilo. One fourth the original size.



Experiment XXIX a. 15 kilo dog. Injection of DEUTEROGELATOSE (K). 0.7 gram per kilo, preceding injection of heteroalbumose. (Cf. tracing *Exper. XXIX b.*) One fourth the original size.



Experiment XXIX b. 15 kilo dog. Cannula in thoracic duct. Injection of HETEROALBUMOSE (H). 0.1 gram per kilo after previous injection of 10.5 grams deuterogelatose (K). (Cf. tracing of *Exper. VI.*) One fourth the original size.

Influence on blood coagulation. — The older literature on the influence of so-called "peptone" on blood coagulation is too familiar to physiologists to require detailed consideration here. Briefly stated, the researches[1] made have demonstrated (*a*) that "peptone" does not of itself possess any clot-preventing power, *i. e.*, the action is an indirect one; and (*b*) that accordingly the clot-preventing power must be attributed to some substance formed within the organism, and possessing a peculiar influence on the blood. Furthermore, it seems probable that the peculiar substance is not a transformation product of the "peptone" itself, but is rather a result of other changes taking place in the body.[2] More recently, through the researches of

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[1] SCHMIDT-MÜLHEIM: Archiv f. Physiol., 1880, p. 30; FANO: *Ibid.*, 1881. p. 277; POLLITZER: Journal of physiology, 1886, vii, p. 283; CAMPBELL: Studies from the Biological Laboratory, Johns Hopkins University, 1887, iv, p. 1; GROSJEAN: Archives de biologie, 1892, xii; LEDOUX: *Ibid.*, 1896, xiv, p. 63.

[2] Cf. CAMPBELL: *Loc. cit.*

Contejean,[1] Gley and Pachon,[2] and especially through the researches of Delezenne,[3] the seat of formation of the anti-coagulating substance has been delegated to the liver, and the important rôle which the leucocytes play in this action has been demonstrated by Delezenne (1898). The explanation formulated at present is as follows: It has long been known that the intravenous injection of "peptone" leads to a pronounced disintegration of leucocytes. Delezenne assumes that this process is accompanied by the liberation of two classes of products, the one accelerating and the other inhibiting blood coagulation. As types of such bodies, the researches of Lilienfeld[4] and others have indicated nucleoproteids, which give rise to intravascular clotting, and histon, a peculiar peptone-like body occurring in leucocytes in the compound nucleo-histon. When these two classes of compounds (clot-retarding and clot-accelerating substances) are formed incidental to the breaking down of the leucocytes, the liver in its protective rôle preserves the organism from destruction through intravascular clotting by combining with the clot-accelerating substance. A condition thus results in which the blood loses completely or partially its power of coagulating on withdrawal from the vessels. The relative rôle of liver and leucocytes is demonstrated by the fact that if a solution of peptone is passed through an excised dog's liver a fluid results which readily produces "peptone" effects when injected into the circulation of a dog. If, however, the liver is previously freed from blood by lavage through the vessels no clot-retarding fluid can be obtained in the manner described. Delezenne's theory therefore implies the existence of two substances antagonistic in their influence on blood coagulation, — a condition which Spiro and Ellinger[5] had previously regarded as probable. These latter investigators observed that the injection of acids or of antipeptone overcomes the clot-retarding power of albumoses, while Dastre and Floresco[6] had previously called attention to the increased alkalinity of "peptone plasma" and the influence of neutralization in inducing coagulation. Lastly, there is evidence that so-called "peptone" may under some conditions hasten the coagulation of the blood. Thus, Thompson[1] has pointed out that with doses of Witte's "pepton" above 2 centigrams per kilo coagulation is retarded. With doses from 7 milligrams to 2 centigrams per kilo coagulation may be considerably accelerated.

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[1] CONTEJEAN: Archives de physiologie, 1895.

[2] GLEY and others: *Ibid.* It is to be noted, however, that Starling (Journal of physiology, 1895, xix, p. 15) has not been able to confirm part of these experiments.

[3] DELEZENNE: Archives de physiologie, 1897, p. 646; 1898, p. 508.

[4] LILIENFELD: Zeitschr. f. physiol. Chem., 1895, xx, p. 89.

[5] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 121.

[6] DASTRE and FLORESCO: Archives de physiologie, 1897, p. 216. Also FLORESCO: *Ibid.*, 1897, p. 777.

Our experiments on this subject were intended to throw light not so much upon the direct mechanism by which the variations in clotting power are produced, as upon the relative effectiveness of various proteid and albuminoid products. A large number of substances, for the most part extracts of various animal tissues or organs, are known to have the power of retarding blood coagulation either *in vivo* or *in vitro*, or both. Grosjean observed that the proteoses from blood-fibrin retard, or even prevent coagulation indefinitely. This action is not produced *in vitro*.[2] True peptone, he found, never acts as vigorously as the proteoses, and confines its influence to a temporary retardation. In either case the lack of coagulation is very rapidly manifested. Thompson[3] has recorded similar results with purified peptone. Deuteroproteose gave him inconstant results, while antipeptone hastened blood coagulation, in conformity with the observations of Spiro and Ellinger.[4] Arthus and Huber[5] have found that much larger doses of gelatoses (2 grams) and caseoses (1.5 grams) per kilo are necessary than in the case of fibrin-proteoses to bring about an absence of coagulation. Lastly, a few experiments have been reported on the action of the albumoses and peptone formed by papaïn digestion.[6]

The accompanying table, giving the results of our own experiments, shows the time required for the complete coagulation of 2 c.c. blood withdrawn from the femoral or carotid artery at various intervals, expressed in minutes or hours. The normal clotting-time given is in every case the average of two or more observations previous to the injection of the substance under investigation.

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[1] THOMPSON: Journal of physiology, 1896, xx, p. 455.

[2] Cf. CAMPBELL.

[3] THOMPSON: Report of Secretary, *loc. cit.*

[4] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 121. See also the older experiments of Pollitzer and of Fano with tryptone.

[5] ARTHUS and HUBER: Archives de physiologie, 1896, p. 865.

[6] CHITTENDEN, MENDEL, and McDERMOTT: This journal, 1898, i, p. 255.

TABLE SHOWING EFFECTS UPON THE COAGULATION OF THE BLOOD.[1]

Experiment.	Weight of animal; kilos.	Grams per kilo injected.	Nature of the substance employed.	Normal coagulation.	Just after injection.	5 minutes after.	15 minutes after.	30 minutes after.	1 hour after.	1½ hours after.	2 hours after.
I	8.8	0.3	Antialbumoses (B) .	6	8	—	—	—	—	—	—
II	6.2	0.3	" " .	6	16	—	—	20h		20	10
III	11.3	0.3	Antialbumoses (C) .	2	—	—	—	—	—	—	—
IV	6.0	0.3	" " .	12	—	—	—	—	—	—	—
V	18.0	0.3	Hemialbumoses (G) .	2	16h	—	—	—	—	—	—
VI	7.5	0.18	Heteroalbumose (H) .	4	—	—	—	—	—	—	—
VII	9.8	0.2	" " .	4	—	—	—	—	—		
VIII	7.0	0.3	Antialbumid (A) . .	6	6	14h	1½h	1½h	45		
IX	6.0	0.3	" " . .	2	13	16h	—	16h			
X	5.4	0.3	" " . .	6	40	2h	30	30	7		
XI	7.6	0.14	" " . .	5	30	16	—	16h	16h		
XII	14.0	0.15	" " . .	4	57	70	27	17	9		
XIII	30.0	0.03	" " . .	3	9	16	25	45	14		
XIV	8.4	0.17	" " . .	1	6	4	8	13	6	9	8
XV	6.8	0.07	Antialbumid (F) . .	3	4	9	4	5			
XVI	9.0	0.3	Antipeptone (D) . .	3	3	3	2	3	3		
XVII	6.5	0.3	" " . .	5	5	8	10	6	10		
XVIII	9.0	0.3	" " . .	4	4	5	5	4	3		
XXXI	4.5	0.45	" " . .	2	4	3	4	10	6		
XIX	11.2	0.3	Hemipeptone (I) . .	9	1	3	3	4	5		
XX	6.5	0.3	" " . .	4	2	3	4	9	7		
XXI[2]	13.5	0.5	" " . .	3	3	4	6		2		
XXII	6.4	1.0	" " . .	4	4h	—	—	—	—	—	
XXIII	5.0	0.3	Albumose-like body (M)	9		6	35	37	20	14	
XXIV	5.0	1.5	Protogelatose (J) . .	10	4	5	6	4			
XXV	4.2	0.3	Gelatin-peptone (L) .	4	3	2	3	4	5		
XXVI	6.0	0.6	" " .	3	2	2	4	4	3		
XXVII	21.0	0.35	Deuterogelatose (K) .	2	2	2		1			
XXVIII	7.0	0.5	" " .	3	3h	3h		20	3	2	
XXIX[2]	15.0	0.7	" " .	3	4	2	8	2	2	2	
XXX	6.5	1.0	" " .	5	2h	—	—	3h	15	4	

[1] The figures in the table denote the number of minutes before coagulation, except when followed by *h*, when they denote hours. All samples marked — were still fluid after standing 24 hours.

[2] The lack of any noticeable action upon the time of coagulation in these two experiments is due to the fact that the lymph was withdrawn through a cannula in the thoracic duct. See under *lymph-formation* for explanation.

The data presented show (1) That in doses of 3 decigrams per kilo of body-weight the albumoses, both hemi and anti, irrespective of the mode of formation, immediately cause suspension of coagulation for at least 24 hours, even in blood withdrawn one or two hours after the injection. (Exper. I–VII.)

(2) Antialbumid produces retardation of clotting, the effect being far less marked than in the case of even smaller doses of albumoses, and furthermore requiring some time to reach its maximum effect, the results varying in proportion to the dose employed. (Exper. VIII–XV.[1])

(3) Both hemi- and antipeptone when introduced into the circulation in doses up to 0.5 gram per kilo cause no retardation or even acceleration of coagulation. Larger doses of hemipeptone[2] (1 gram per kilo) however act more like the albumoses. (Exper. XXII.)

(4) Protogelatose, as formed by gastric digestion, tends to hasten coagulation even when introduced in large doses.[3] Large doses of deuterogelatose retard coagulation quite noticeably, our results agreeing in a general way with the observations made by Arthus and Huber[4] with mixed gelatoses and gelatin-peptone.[5] True gelatin-peptone entirely free from gelatoses tends to accelerate slightly the coagulation of the blood.

Independence of arterial pressure and coagulation phenomena. — The results of the present experiments lend weight to the view that the characteristic fall of arterial pressure and the accompanying lack of coagulability are entirely independent phenomena. The former is doubtless due to a direct and immediate action of the peptones and proteoses on the neuro-muscular apparatus of the vessels, while the latter phenomenon stands in no causal relation to the arterial fall. Thus we have observed in many experiments an entire absence of diminished coagulability, notwithstanding a pronounced fall in pressure. (Cf. experiments on peptone, gelatoses, and gelatin-peptone.)[1] Ledoux[2] has arrived at a similar conclusion, and Thompson[3] has found that a dose of Witte's "pepton" entirely insufficient to retard coagulation may still lower blood pressure, — as Fano[4] had previously found to be the case in general with acid-albumin. Finally, Dastre and Floresco[5] have quite recently demonstrated that intravenous injections of bile may entirely prevent the action of proteoses on blood coagulation without interfering with the fall of arterial pressure.

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[1] With *antialbumid* (E) *i. e.*, a more insoluble form of the substance separating as a gelatinous coagulum when antialbumid (A) was subjected to pancreatic digestion, some peculiar results were obtained which are not included in our table. Thus, in one experiment a dog weighing 5.2 kilos received an injection of 25 c.c. of a solution of this antialbumid in weak ammonia (containing 0.29 gram of the dry antialbumid). Three blood samples taken before the injection clotted in two minutes, and arterial pressure stood at 90 mm. of mercury. One minute after the injection blood pressure fell to 10 mm. and two minutes later the animal was dead. The heart and larger blood vessels were opened and the blood was found to be clotted solid. A like result was obtained with another dog of the same body-weight, by using a somewhat larger dose of the antialbumid, namely, 0.58 gram. In a third experiment, with a dog of 13.6 kilos, using 0.35 gram of antialbumid as the total amount injected, there was a marked fall of arterial pressure, but no effect on blood coagulation. Lastly, with a fourth dog of 16 kilos having a cannula in the thoracic duct, the injection of the antialbumid (0.29 gram in 25 c.c. fluid) led to a fall of arterial pressure, a retardation of 10–12 minutes in the coagulation of the blood, a cessation of the flow of urine from the kidneys, with a small increase in the flow of lymph.

[2] Cf. the experiments on papaïn-peptone by CHITTENDEN, MENDEL, and McDERMOTT: This journal, 1898, i, p. 271.

[3] Cf. DASTRE and FLORESCO: Archives de physiologie, 1896, p. 402; also FLORESCO: *Ibid.*, 1897, p. 777.

[4] ARTHUS and HUBER: Archives de physiologie, 1896, p. 857.

[5] With reference to the peculiar results obtained in Exper. XXXI see the section on lymph.

Immunity. — Since the experiments of Schmidt-Mülheim[6] and more particularly of Fano,[7] it has been known that an intravenous injection of so-called "peptone" (propeptones) which suffices to render the blood non-coagulable for a time apparently confers upon the animal a degree of immunity against subsequent injections of the "peptone." For example, if after the return of normal coagulability to the blood a second injection of albumoses is made, the latter now fails to deprive the blood of its ordinary extravascular clotting power. Furthermore, Fano demonstrated that an injection of tryptone (antipeptone from pancreatic digestion) which is without influence on the coagulation of the blood, nevertheless may render the dog immune against subsequent injections of albumose-peptone. Spiro and Ellinger[8] have likewise found that injection of antipeptone has an antagonistic influence on the action of Witte's "pepton" on blood coagulation. Grosjean[9] in his comparative study of the physiological action of propeptone and peptone reached the following conclusions: A second injection of propeptone is without effect on blood coagulation if it be carried out on an animal (dog) in which the effect of a previous injection has just disappeared. This is true, says Grosjean, even when the second injection is made with a large dose of the propeptone. The immunity acquired persists, for the most part, about twenty-four hours after the primary return of coagulability, when the blood again becomes coagulable; the time of coagulation not only returns to the normal but may even be shortened. With reference to purified peptone Grosjean concludes that the injection of this substance may likewise confer immunity against subsequent injections of both peptone and propeptone.

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[1] See also ARTHUS and HUBER: *loc. cit.*, p. 861.

[2] LEDOUX: Archives de biologie, 1896, xiv, p. 63.

[3] THOMPSON: Journal of physiology, 1896, xx.

[4] FANO: Archiv f. Physiol., 1881, p. 277.

[5] DASTRE and FLORESCO: C. r. de la soc. de biologie, 1898, p. 458.

[6] SCHMIDT-MÜLHEIM: Archiv f. Physiol., 1880, p. 30.

[7] FANO: Archives ital. de biologie, 1882, ii, p. 146.

[8] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 137.

[9] GROSJEAN: Archives de biologie, 1892, xii.

Gley and Le Bas[1] have also carried out a series of experiments on the behavior of the blood with reference to its coagulability after repeated injections of Witte's "pepton." As a result of their systematic study on a large number of dogs they conclude that in general the period of time during which the blood remains non-coagulable after a rapid injection of proteoses increases with the dose of proteoses employed per kilo of body-weight. Furthermore, the length of time during which immunity to subsequent injections persists also increases with the original dose. Thus, absolute immunity persists after return of the blood to its normal coagulability, about 15 minutes for 0.01 gram per kilo, and about 5 hours for a dose of 0.3 gram per kilo.[2] The investigations of Gley and Le Bas thus fail to confirm current statements, namely, that a preliminary injection of proteoses may confer immunity for twenty-four hours toward subsequent injections.

Arthus and Huber[3] observed that preliminary injections of gelatoses and caseoses were effective in preventing the anti-coagulating effect of subsequent large doses of the same substance. A second fall of blood pressure, however, was not prevented. Grosjean has pointed out that successive doses of both proteoses and peptones produce successively a less marked fall of arterial pressure, and a second or third injection may even be without effect on this function; a condition attributed to a degree of immunity acquired by the preliminary injection. It is to be noted, however, that in Gros-

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[1] Gley and Le Bas: Archives de physiologie, 1897, p. 848. The experiments of Contejean (*Ibid.* 1895) are not referred to above since they have only an indirect bearing on this subject.

[2] Gley and Le Bas: *loc. cit.*, p. 860.

[3] Arthus and Huber: Archives de physiologie, 1896, p. 857.

TABLE OF RESULTS BEARING UPON SO-CALLED "PEPTONE" IMMUNITY.

Experiment.	Weight of animal: kilos.	First Injection.		Time between first and second injection.	Second Injection.		Coagulation of the Blood.				Arterial Pressure.		
		Grams per kilo injected.	Nature of the substance employed.		Grams per kilo injected.	Nature of the substance employed.	Prior to second injection.	5 minutes after	15 minutes after.	30 minutes after.	Prior to second injection.	5 minutes after	15 minutes after.
I	8.8	0.3	Antialbumoses (B)	2¾h	0.3	Witte's peptone	10	20	30		110	60	100
VIII	7.0	0.3	Antialbumid (A)	50	0.3	" "	45				90	22	
X	5.4	0.3	" "	2h	0.3	Antialbumid (A)	9	1h			130	120	150
X			" "	20	0.3	Witte's, 3d injection		30			150	75	135
XV	6.8	0.07	Antialbumid (F)	45	0.3	Witte's peptone	5	10			100	25	40
XVI	9.0	0.3	Antipeptone (D)	1½h	0.25	" "	3	1½h	—	27	160	50	120
XX	6.5	0.3	Hemipeptone (I)	1h	0.1	Heteroalbumose (H)	7	5	4	8	130	55	75
XXI	13.5	0.5	" "	2¼h	0.2	" "	2	3	2		145	85	110
XXIV	5.0	1.5	Protogelatose (J)	6h	0.1	" "	4		—		130	55	100
XXVI	6.0	0.6	Gelatin-peptone (L)	1½h	0.3	Witte's peptone	2	4	—		115	30	60
XXIX	15.0	0.7	Deuterogelatose (K)	2½h	0.1	Heteroalbumose (H)	2	6	6		150	60	80

NOTE. Figures in the table referring to time denote minutes, except when followed by h, when they denote hours. Under arterial pressure, the figures denote millimetres. Under coagulation of blood, samples marked — were still fluid twenty-four hours after the experiment. The numbers of the experiments correspond with those given in the preceding tables.

jean's experiments the injections were undertaken at relatively brief intervals.

In the preceding table are presented protocols from a few of our own experiments bearing on the problem of peptone-immunity. The data presented are somewhat incomplete and fragmentary, still a few facts are clearly demonstrated. Thus it is seen that the injection of active proteoses, such as pure heteroalbumose or the mixed proteoses present in Witte's "pepton," within an hour or two after injection of the substances enumerated in the table fails to develop the characteristic clot-preventing power of the blood. There is to be sure some slight retardation of coagulation, thus indicating that the so-called immunity is by no means complete; but the effect produced by the later injections is very slight indeed as compared with the ordinary action of these products. Experiments XX, XXII, XXIX, XV, and I afford evidence of the truth of this assertion. In other cases, as in Experiments XVI and XXVI, the immunizing effect is manifested by a delay in the appearance of retarded coagulation. Thus in the two experiments just referred to, a considerable period elapsed before the blood withdrawn from the vessels assumed the characteristics of "peptone-blood." It would almost seem as if the results indicated a deficiency of the clot-preventing substances in the body after the preliminary injection, and that the latter are only *slowly* produced under the influence of the second injection.

With reference to the effects of a second injection of proteoses on arterial pressure, our results clearly show that mean arterial pressure is lowered somewhat by the second injection, thus indicating that immunity in this direction is by no means complete. That the first injection, however, does give rise to some degree of immunity is clearly shown by the fact that the second fall of pressure is noticeably less than the first one, and by the fact that the return of pressure to the normal follows somewhat more rapidly than after the first injection. Our results thus confirm, in part, Grosjean's observations, and extend them by demonstrating that the immunity acquired is by no means characteristic of specific products *injected*, but rather due to a specific reaction produced in each case within the organism itself. Delezenne[1] has arrived at a similar conclusion from quite different observations. Lastly, Dastre and Floresco[2] have recently shown that the power of conferring immunity to "peptone" injections is common to a large number of substances, *i. e.*, urine, bile, salts of iron, calcium chloride, etc.

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[1] DELEZENNE: Archives de physiologie, 1897, pp. 657-659.

[2] DASTRE and FLORESCO: C. r. de la soc. de biologie, 1898, p. 457.

Influence on lymph-flow, etc. — Since Heidenhain's[1] masterly researches on the action of lymphagogues there has been, so far as we recall, no attempt to compare the lymphagogic action of the individual proteoses and peptones. It is well known that various substances which prevent clotting of the blood simultaneously increase the flow of lymph from the thoracic duct; the substances studied, however, have been for the most part merely extracts of various organs and tissues (leech-extract, extracts of crayfish and mussels). A systematic study of the active constituents of these extracts would be of great interest.

The following table contains the results of several of our experiments on the action of certain specific proteid and albuminoid derivatives on the flow and composition of the lymph. All the animals experimented on were dogs, and were deprived of food for 24–36 hours prior to the experiment.

The results of our experiments justify the following conclusions:

(1) Antialbumid, hemipeptone, heteroalbumose, and deuterogelatose may act as true lymphagogues. This is demonstrated not only by the marked increase produced in the flow of lymph from the thoracic duct, but likewise by the characteristic changes resulting in the composition of the fluid, namely, increase in total solids, the ash-content remaining constant.[2] In every case where an increased flow of lymph occurred, the fluid grew slightly reddish in color and its clotting was markedly delayed, — two phenomena characteristic of "lymphagogic" lymph.

(2) In every case where the lymph was thus diverted from the general circulation the clotting time of the blood was only slightly, if at all, affected by the injection, while the coagulability of the lymph was delayed. This agrees with the observations of Gley and Pachon,[3] who announced that the ligature of the lymphatics of the liver prevents the ordinary clot-retarding action of albumose injections in dogs; and with the observations of Spiro and Ellinger, similar to

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[1] Heidenhain: Archiv f. d. ges. Physiol., 1891, xlix, p. 216.

[2] Cf. Heidenhain: *loc. cit.*

[3] Gley and Pachon: C. r. de l'acad. des sciences, 1895, p. 383; Archives de physiologie, 1895, p. 711; C. r. de la soc. de biologie, 1895, p. 741; *Ibid.*, 1897. Cf. also Starling: Journal of physiology, 1895, xix, p. 15; Delezenne: Archives de physiologie, 1896, p. 657.

TABLE SHOWING INFLUENCE ON LYMPH-FLOW, ETC.[1]

EXPERIMENT.	TIME.	Lymph-flow in 10 min.; c.c.	Total solids of lymph; per cent.	Ash of lymph; per cent.	Clotting time of lymph; min.	Clotting time of blood; min.	Arterial pressure in mm. Hg.	Urine flow in 30 min.; c.c.
Antialbumid (A) [Exper. XII.] Dog of 14 kilos. Injection of 0.15 gram per kilo body-weight.	Immediately before injection . . .	1.2	4.90	0.85	10	4	140	
	Immediately after injection . . .	10.0	6.73	0.85	?	57	90	
	One half hour after injection . . .	3.0	6.10	—	?	17	130	
Hemipeptone (I) [Exper. XXI.] Dog of 13.5 kilos. Injection of 0.5 gram per kilo body-weight	Immediately before injection . . .	4.5	4.47	0.87	10	3	130	4.0
	Immediately after injection . . .	17.0	5.60	0.86	180	3	40	15.0
	One half hour after injection . . .	5.0	4.81	—	1	4	130	
HETEROALBUMOSE (H)[2] 0.2 gram per kilo injected; 2¼ hours after the injection of hemipeptone.	Immediately after second injection .	10.5	—	—	—	3	85	none
Deuterogelatose (K) [Exper. XXVII.] Dog of 21 kilos. Injection of 0.35 gram per kilo body-weight.	Immediately before injection . . .	4.0	5.77	0.85	30	2	115	1.0
	Immediately after injection . . .	8.6	5.69	0.89	15	2	115	10.0
	One half hour after injection . . .	4.7	4.77	—	15	½	120	
Deuterogelatose (K) [Exper. XXIX.] Dog of 15 kilos. Injection of 0.7 gram per kilo body-weight.	Immediately before injection . . .	3.0	6.58	0.92	10	3	130	5.0
	Immediately after injection . . .	15.0	7.78	0.93	90	4	60	15.0
	One half hour after injection . . .	7.2	6.24	—	20	2	150	7.0
HETEROALBUMOSE (H)[2] 0.1 gram per kilo injected; 2½ hours after the injection of deuterogelatose.	Immediately after second injection .	15.0	7.35	—	50	6	60	none

Heteroalbumose (H)
[Exper. VII.] A dog of 9.8 kilos received an injection of 0.2 gram per kilo body-weight. This was followed by a pronounced fall of arterial pressure; the blood samples taken one hour after the injection failed to clot in twenty-four hours. Owing to an accident to the cannula the lymph-flow could not be measured. There was, however, a pronounced increase after the injection.

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[1] The experiments are numbered to correspond with the numbers used in the other tables.

[2] Second injection.

our own, on dogs with the thoracic duct cannulized.[1] In other words, the observations lend favor to the view that the anti-clotting substance is formed in the liver and reaches the blood mainly through the lymph.

(3) A second injection gives rise to a marked renewal of lymph-flow, independent of any action on blood coagulation. The two Experiments XXI and XXIX do not, however, permit the immediate conclusion that the lymphagogic action and clot-preventing phenomena are entirely independent,[2] inasmuch as the diversion of the lymph from the circulation may prevent the anti-coagulating substance from reaching the blood.[3] Since some dogs are naturally immune to albumose injections, no far reaching conclusions can be drawn from the data at hand.

(4) Ordinary doses of deuterogelatose (0.3 gram per kilo) give rise to no marked lymphagogic action, large amounts being required to produce characteristic effects. This corresponds with the other observations made on the physiological action of this substance. With heteroalbumose, lymphagogic action is as strongly marked as the action on blood coagulation and arterial pressure already noted.

(5) Previous injection of hemipeptone or deuterogelatose is entirely without influence on the lymphagogic action of heteroalbumose; the latter producing a marked increase in the flow of lymph when injected a short time after the action of the primary substances has ceased.

Influence on the flow of urine, etc. — In conformity with previous observations our results, shown in the following table, lead to the conclusion that the albumoses, owing to their marked influence in lowering blood pressure, tend to cause a complete cessation of the flow of urine. With heteroalbumose, however, the results seemingly justify a somewhat different conclusion. Thus, in three experiments, (XXI, XXIV, and XXIX) some hours after the animals had received injections of substances which act as diuretics and lymphagogues, an injection of heteroalbumose was given. This resulted in a complete stoppage of the flow of urine; not a drop of urine was obtained within the half hour or more after this injection, *although the fall of arterial pressure was comparatively slight and transitory.* Hence, it follows that heteroalbumose, unlike the other albumoses, must exert some influence upon the action of the kidneys independent of blood pressure; possibly, a specific influence upon the secretory activity of the epithelial cells. In any event heteroalbumose may check the formation of urine even when arterial pressure is not materially affected. On the other hand, as already pointed out, heteroalbumose stimulates in marked degree the flow of lymph. (See preceding table on lymph-flow.)

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[1] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

[2] Cf. STARLING: Journal of physiology, 1893, xiv, p. 140; also GLEY and PACHON: Archives de physiologie, 1897, p. 861.

[3] ASHER and BARBÈRA (Zeitschr. f. Biologie, 1898, xxxvi, p. 154) have put forward the opinion that lymphagogues are substances which stimulate the activity of various organs and especially glands. In agreement with this view they find that "peptone" is an active "cholagogue." Starling and others, however, have questioned this conclusion. See Proceedings of Fourth International Physiological Congress. Centralbl. f. Physiol., 1898, xii.

Antialbumid acts much like ordinary albumoses in checking the secretion of urine, but if comparison is made, by reference to the table showing arterial pressure, of the influence on renal secretion and mean arterial pressure, as in Experiments VIII, X, and XI, it will be seen that the inhibitory effect on the secretory action of the kidneys is considerably greater than would naturally be expected from the short period of diminished arterial pressure. This fact obviously suggests a specific action on the part of antialbumid upon the kidneys independent of pressure.

On the other hand, large doses of hemipeptone, protogelatose, and deuterogelatose have a marked diuretic effect, increasing not only the volume of urine secreted in a given time, but likewise the content of solid matter, as shown by the very decided increase in specific gravity.[1] Coincident with the diuresis it will be observed that mean arterial pressure is slightly raised under the influence of the substance. The marked rise in the specific gravity of the urine, noticed after the injections, is associated mainly with the rapid excretion of the substance thrown into the circulation. As has been previously noted[2] in this connection, a primary proteose introduced into the blood is eliminated, in part at least, as a secondary proteose. In other words, the primary body undergoes hydration in the kidney[3] or elsewhere, and is excreted through the urine in slightly altered form. Thus, in Experiment XXIV with protogelatose not only was the rate of flow

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[1] See CHITTENDEN: Science N. S. v, June 11, 1897, p. 902; also THOMPSON: Journal of physiology (Proceed. Physiol. Soc.), 1898, xxii, p. xi.

[2] NEUMEISTER: Zeitschr. f. Biologie, 1887, xxiv, p. 272; also CHITTENDEN, MENDEL, and McDERMOTT: This journal, 1898, i, p. 266.

[3] NEUMEISTER: *loc. cit.*

TABLE SHOWING EFFECTS UPON URINARY SECRETION.

Experiment.	Weight of animal; kilos.	Grams per kilo injected.	Nature of the substance injected.	Urine in 30 mins. before injection.		Urine in 30 mins. after injection.		Remarks.
				Vol.	Sp. gr.	Vol.	Sp. gr.	
				c.c.		c.c.		
VIII	7.0	0.3	Antialbumid (A) . . .	19.2		2.6		
X	5.4	0.3	" " . . .	7.2		0		
XI	7.6	0.14	" " . . .	7.0		2.9		
XVI	9.0	0.3	Antipeptone (D) . . .			30.0		Strong biuret reaction.
XVII	6.5	0.3	" " . . .	6.7		10.5		" " "
XVIII	9.0	0.3	" " . . .	4.2	1026	43.5	1016	" " "
XIX	11.2	0.3	Hemipeptone (I) . . .	46.2	1031	38.0	1036	Urine almost black.
XX	6.5	0.3	" " . . .	4.0		4.0		No biuret; normal color.
XXI	13.5	0.5	" " . . .	4.0	1067	15.0	1042	No biuret; dark color.
XXII	6.4	1.0	" " . . .	17.5	1035	55.0	1035	" " "
XXIII	5.0	0.3	Albumose-like body (M)	2.5		0		" " "
XXIV	5.0	1.5	Protogelatose (J) . . .	13.8	1021	32.0	1061	Dark color. Contained deuterogelatose and peptone.
XXV	4.2	0.3	Gelatin-peptone (L) . .			25.0		No biuret; dark color.
XXVI	6.0	0.6	" " " . .	15.0	1046	10.0	1045	" "
XXVII	21.0	0.35	Deuterogelatose (K) . .	1.0		10.0		Dark urine. Contained deuterogelatose and peptone.
XXVIII	7.0	0.5	" " . .	8.0	1046	10.0	1093	
XXIX	15.0	0.7	" " . .	5.0	1062	15.0	1093	
XXX	6.5	1.0	" " . .	2.1		4.5		

of the urine more than doubled, but the specific gravity rose from 1021 to 1061. Examination of the urine showed the presence of a large amount of gelatose, but this was mainly deuterogelatose. Further, on saturating the urine with ammonium sulphate under appropriate conditions for removal of all the gelatoses, and testing the filtrate by the biuret test, a distinct reaction for true gelatin-peptone was obtained. Five and a half hours after the injection the urine still contained an appreciable amount of gelatose. After injection of deuterogelatose the urine was likewise found rich in gelatin-peptone, while some unaltered gelatose was also found. This excretion of gelatoses through the urine as gelatoses and gelatin-peptone was quite marked, and we were able to separate from such urines several grams of fairly pure gelatose, thus showing that the elimination through this channel was not inconsiderable. If it is true, as suggested by Neumeister, that the hydration of a primary proteose to secondary proteose and the hydration of the latter to true peptone in these cases is due to the presence of pepsin in the kidneys acting in conjunction with acid temporarily present, then our results testify to the extreme vigor of such action, and indirectly suggest the presence of considerable active enzyme. Whether this hydrolytic action is truly due to the presence of an enzyme our results afford no answer. In this connection it will be remembered that Hofmeister[1] observed that when amphopeptone was injected subcutaneously four fifths of the substance appeared in the urine unaltered. Neumeister[2] likewise noted that amphopeptone was excreted as such through the urine. In a paper from this laboratory,[3] however, attention was called to the fact that peptone formed through the action of papaïn was much less markedly eliminated through the urine than deuteroalbumose; this statement being based upon the much weaker biuret reaction given by the urine. In conformity with this observation we have noted in the present experiments with hemipeptone and gelatin-peptone that after the intravenous injection of these substances their presence in the urine could not be detected by the biuret reaction. Whether this fact is to be taken as an indication of their excretion by the urine in the form of amido-acids, etc., formed through proteolytic action in the kidneys, or whether it is due to decomposition taking place elsewhere or to a storing up within the body we cannot say.

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[1] Hofmeister: Zeitschr. f. physiol. Chem., 1881, v, p. 131.

[2] Neumeister: Zeitschr. f. Biologie, 1887, xxiv, p. 287.

[3] Chittenden, Mendel, and McDermott: This journal, 1898, i, p. 275.

OBSERVATIONS ON THE CHEMICAL NATURE AND GENERAL PROPERTIES OF THE PROTEID CLEAVAGE PRODUCTS USED IN THE PRECEDING EXPERIMENTS.

Among the many earlier results obtained in the attempt to throw light upon the chemical nature of the proteid molecule those recorded by Schützenberger[1] were particularly suggestive, especially from a physiological standpoint. The breaking down of the proteid molecule into two equal parts by the action of 3 to 5 per cent sulphuric acid at 100°C. naturally suggested the possible existence of two distinct groups in the mother proteid; a suggestion which was intensified by the observations of Kühne[2] that these two cleavage or alteration products behaved quite differently toward alkaline pancreatic juice. Schützenberger through his hydrolysis of coagulated egg-albumin by dilute sulphuric acid obtained an insoluble residue equal to about 50 per cent of the original proteid, to which he gave the name of hemiprotein, while the 50 per cent of soluble matter was represented by a row of substances among which leucin, tyrosin, peptone, and albumoses (as now termed) were conspicuous. As Schützenberger pointed out, it was quite evident that these latter products resulting from this hydrolysis represented a half of the proteid molecule much less stable than that portion of the proteid from which the so-called hemiprotein was derived. This more readily decomposable half of the proteid he considered as represented by a body which he termed hemialbumin, and which by continued hydrolysis gave rise to peptone and amido-acids. These conclusions were verified in part, and extended by Kühne in his study of the action of the proteolytic enzymes upon these two bodies; *i. e.*, hemiprotein and hemialbumin. The former was found to be slowly digestible by pepsin-hydrochloric acid, while by alkaline pancreatic juice it was eventually transformed in great part into a peptone without any trace of amido-acids being formed.[3] In other words, the substance was remarkably resistant to that secondary action of the pancreatic enzyme by which pancreatic proteolysis is especially characterized. On this account Kühne suggested the name of antialbumid as more expressive of the general nature of the substance than the term hemiprotein, and by this name the substance has generally been known for the past twenty years. The so-called hemialbumin of Schützenberger was found by Kühne to be readily digestible in artificial gastric juice, giving rise to peptone, while by the action of pancreatic juice leucin and tyrosin were formed in abundance. To the hemialbumin of Schützenberger, Kühne gave the name of hemialbumose, which prevailed for a time until it was found that this substance was in reality a mixture of several related substances, to which the name of albumoses was applied.[1] Through these researches, followed by many others that need not be enumerated here, the view that in the native proteid molecule there are present two distinct atomic complexes has gradually gained credence, and as a result the prefixes hemi and anti have been, and are, widely used to designate inner differences in the various hydrolytic cleavage products of the proteids, whether formed by the action of boiling dilute acids or by the action of proteolytic enzymes. A hemi body has thus come to mean a substance which under the influence of trypsin will break down into crystalline amido-acids and other simple products, while an anti body fails to yield any such simple products under the action of trypsin. The researches of Neumeister[2] and others have shown, however, that when this crucial test is applied to the specific proteoses formed in gastric digestion, for example, there is invariably an admixture of so-called hemi and anti groups; not an equal mixture, — for protoproteose is apparently composed in great part of hemi groups with only a small admixture of anti groups, while heteroproteose is made up chiefly of anti groups, as judged by the behavior of the respective bodies toward trypsin. In the amphopeptone of gastric digestion, on the other hand, we have apparently a commingling in equal proportion of hemi and anti groups, for if this substance is digested for some time with a vigorous pancreatic juice, approximately one half breaks down into crystalline decomposition products, leaving a residue of peptone resistent to the further action of trypsin, *i. e.*, an antipeptone. How far these terms hemi and anti are to be considered as representing real differences in chemical constitution is very uncertain. It may be that the differences are more apparent than real, and that the characteristic behavior toward trypsin is the result of more or less superficial peculiarities. Granting that this is so, the terms still have significance as indicating physiological differences of some moment and are thus of value. Certainly the view at one time suggested that an anti body contained no aromatic groups capable of yielding tyrosin under any conditions is not tenable. To be sure, a pure antipeptone gives little or no Millon's reaction, but antialbumid boiled for some time with 10 per cent sulphuric acid will yield considerable tyrosin, although when acted upon by an alkaline solution of trypsin no tyrosin whatever results. Thus, Dr. Alice H. Albro, working in this laboratory with antialbumid made from coagulated egg-albumin, found on boiling the substance with 10 per cent sulphuric acid for 80 hours that 2.7 per cent of tyrosin was formed together with considerable leucin. In this connection it will be remembered that Erlenmeyer and Schöffer obtained 1 per cent of tyrosin by boiling egg-albumin with sulphuric acid, while Schützenberger obtained 2.0 per cent by heating egg-albumin at 160–200° C. with baryta water for 4 to 6 days.[1] Obviously, one cannot draw very sharp comparisons between these several results, but it is quite clear that antialbumid made from coagulated egg-albumin by the action of 4 per cent sulphuric acid at 100° C. yields fully as much tyrosin on decomposition with a strong mineral acid as the mother proteid. Hence the difference in the result when these two substances are treated with active pancreatic juice must be due, not to the lack of the characteristic aromatic group in the antialbumid molecule, but rather to some peculiar state of combination of the atoms or radicles which prevents the formation of tyrosin or other amido-acid through the action of trypsin. In the case of antialbumid formed by the hydrolytic action of a dilute acid it might perhaps be claimed that it is purely an artificial product, and that its resistance to the secondary action of trypsin is merely the result of some slight alteration of the original proteid attendant upon the process of cleavage. As opposed to this idea, however, we have the well-known fact that the original coagulated albumin placed directly in alkaline pancreatic juice breaks down step by step to leucin, tyrosin, etc., leaving at the end a large amount — perhaps 50 per cent — of a peptone (antipeptone) which resembles the peptone resulting from the pancreatic digestion of antialbumid. In other words, there is abundant reason for believing that the so-called anti group is contained as such in the native proteid, and that in the cleavage with dilute sulphuric acid it is split off as antialbumid, while in the pancreatic digestion of the native proteid it is isolated as antipeptone through conversion of the so-called hemi groups into amido-acids and nitrogenous bases.

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[1] SCHÜTZENBERGER: Bulletin de la Société chimique de Paris, 1875, xxiii, and xxiv.

[2] KÜHNE: Verhandl. d. Naturhist.-Med. Vereins zu Heidelberg, 1877, i, p. 236; KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1883, xix, p. 159.

[3] KÜHNE and CHITTENDEN: *loc. cit.*

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[1] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1884, xx, p. 11.

[2] NEUMEISTER: Zeitschr. f. Biologie, 1887, xxiii, p. 380.

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[1] See Hermann's Handbuch der Physiologie, v (2), p. 212.

While it is thus possible to isolate with some degree of purity bodies belonging to or derived from the anti half of the proteid molecule, less satisfactory is the isolation of the so-called hemi bodies. From the amphopeptone formed in gastric digestion, the antipeptone can be separated through destruction of the hemi groups, but there is no way known of isolating the hemipeptone from this mixture. A method promising better results is the hydrolytic cleavage of the native proteid with dilute acid, accompanied as it is by the splitting off of the anti groups as antialbumid. While this results in the formation of soluble hemialbumoses and hemipeptone, which can easily be separated, it unfortunately happens that the antialbumid formed also undergoes hydration to some slight degree with consequent formation of some soluble antialbumoses and antipeptone. These latter bodies once mixed with the corresponding hemi products cannot be separated, and hence are an ever-present impurity. Still, this method affords the only way, at present, of obtaining the so-called hemialbumoses and peptone, and we have had recourse to it as the only available method, understanding, however, that the several products are by no means wholly free from corresponding anti bodies.

As already stated in the first part of this paper, it seemed very desirable in view of the importance attaching to the physiological action of the various digestive products of the proteids to ascertain as definitely as possible the part played by the several components of the ordinary products of proteolytic action. With this end in view we have devoted particular attention to the anti products, and in order to insure their freedom, so far as possible, from the so-called hemi groups we have made use of antialbumid as the mother substance in the preparation of antialbumoses and antipeptone.

Hydrolysis and cleavage of coagulated egg-albumin with formation of antialbumid, etc. — In obtaining sufficient material for our purpose it was necessary to repeat the process several times, but the general line of procedure in each case was as follows: The whites of twelve dozen eggs were coagulated by pouring them slowly into a large volume of boiling water acidified slightly with acetic acid, the coagulum collected on a cloth filter and washed with large quantities of boiling water. It was then pressed as dry as possible and placed in a large flask with 4 per cent sulphuric acid (360 grams of the moist coagulum in 1200 c.c. of the acid). This mixture was then heated at

100° C. in a large steam sterilizer (Arnold's), with occasional agitation, for 12 to 15 hours, after which the gelatinous antialbumid was separated from the acid solution (containing the hemi bodies) by filtration through paper and freed from all soluble matter by long continued washing with water. The washing was continued until the wash-water reacted only faintly acid. In some cases the antialbumid was again heated at 100° C. for 12 hours with a fresh lot of 4 per cent sulphuric acid. The crude antialbumid was next warmed at 40° C. for forty hours with a large volume of an active solution of pepsin-hydrochloric acid for the complete removal of adherent hemi bodies or of any unaltered egg-albumin. The residual antialbumid was then filtered off and washed thoroughly with distilled water until the washings were free from chlorine. The product was next dissolved in a solution of 0.5 per cent sodium carbonate, the fluid filtered through paper, and the antialbumid reprecipitated by neutralization with 0.2 per cent hydrochloric acid, after which it was again washed with water until free from chlorine, and lastly with 95 per cent alcohol. This product constitutes *antialbumid* (*A*).

In separating the so-called hemialbumoses from the 4 per cent sulphuric acid solution, the clear filtered fluid was neutralized with ammonia and evaporated to a convenient volume, the neutralization precipitate filtered off, and the further concentrated fluid eventually saturated boiling hot with ammonium sulphate in acid, neutral and alkaline reaction according to the method suggested by Kühne.[1] The precipitate of mixed albumoses was dissolved in water, a little thymol added, and the fluid dialyzed in running water until the sulphate was entirely removed. When the dialysis was completed a small quantity of heteroalbumose was found adherent to the parchment. This was therefore filtered off, washed thoroughly with water, lastly with alcohol, and dried. This product constitutes *heteroalbumose* (*H*). Assuming the correctness of Neumeister's statement that heteroalbumose as formed in pepsin-proteolysis is composed mainly of anti groups, it suggests that the formation of this substance in the hydrolysis with sulphuric acid may be taken as a measure of the extent to which in this hydrolysis anti groups are split off in forms other than antialbumid. On the other hand there is perhaps no logical reason why a heteroalbumose might not be formed under this peculiar method of hydrolysis, out of hemi groups mainly.

The above fluid freed by dialysis from ammonium sulphate and heteroalbumose, after filtration from the latter substance, was concentrated to a syrup, precipitated with alcohol, and the precipitate washed thoroughly with hot alcohol and dried. This product constitutes *hemialbumoses* (*G*), and is composed of a mixture of hemi protoalbumose and hemi deuteroalbumose.

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[1] KÜHNE: Zeitschr. f. Biologie, 1892, xxix, p. 1.

The hemipeptone contained in the ammonium sulphate-saturated fluid after separation of the mixed albumoses was obtained as follows: the fluid was concentrated somewhat, cooled, and the crystals of ammonium sulphate separated, this operation being repeated four times, thus accomplishing the removal of a large amount of the contained sulphate. During the repeated heating of this solution a small quantity of a dark, oily, albumose-like substance was obtained, which was filtered off and freed from ammonium sulphate, by the method used in the purification of the peptone, *i. e.*, with barium carbonate, etc. The product so isolated constitutes the *albumose-like body* (*M*). The solution was next treated with barium hydroxide until the larger portion of the sulphate was precipitated, after which the last portions of the ammonium salt were removed by warming the solution with pure barium carbonate. The filtered fluid was then treated with very dilute sulphuric acid, drop by drop, until the last trace of dissolved barium was removed and the filtrate concentrated to a small volume, precipitated with alcohol and the precipitate washed repeatedly with boiling alcohol. This product constitutes *hemipeptone* (*I*).

Formation of antialbumoses by the action of pepsin-acid on pure antialbumid. — When antialbumid is warmed at 40° C. with an active solution of pepsin-hydrochloric acid (0.2 per cent HCl) there results a slow but gradual digestion of the proteid with formation of soluble antialbumoses together with a trace of peptone. Using antialbumid (A) as the mother substance, digestion with pepsin-acid was carried on for some days, the undigested residue filtered off, the solution neutralized with sodium carbonate, concentrated, and the albumoses separated by saturation of the boiling solution with ammonium sulphate. The gummy precipitate so obtained was dissolved in water, dialyzed in running water until free from sulphate, then concentrated to a syrup and precipitated with alcohol. The product was a mixture of protoalbumose and deuteroalbumose, and constitutes the preparation called *antialbumoses* (*B*).

Antialbumoses and antipeptone formed by the action of an alkaline solution of trypsin on antialbumid. — Antialbumid (A) was dissolved in 0.5 per cent sodium carbonate and the solution warmed at 40° C.

for five days with a purified solution of trypsin,[1] also in 0.5 per cent sodium carbonate, thymol being added to prevent putrefaction. After the clear solution had been at 40° C. for a few hours it became opaque and soon formed a thick jelly due to the separation of a portion of the antialbumid in a slightly modified form, as has frequently been described in other places.[2] At the expiration of the five days a large proportion of this insoluble matter had disappeared, but there still remained an appreciable amount of the substance, which was filtered off, thoroughly washed with water, and dried. This constitutes *antialbumid* (*E*). The alkaline solution containing the albumoses and peptone was neutralized with dilute hydrochloric acid, the slight precipitate removed by filtration, and the fluid concentrated to a convenient volume. The antialbumoses and antipeptone were then separated by ammonium sulphate and isolated exactly as described under the head of hemialbumoses and hemipeptone. The antipeptone, however, owing to the presence of sodium chloride, was further purified by dialysis until the chloride was entirely removed. The product is *antipeptone* (*D*).

The antialbumoses, indicated as *antialbumoses* (*C*), were a mixture of proto and deuteroalbumose. This is a matter of some moment chemically, for it is generally assumed that in trypsin-proteolysis primary proteoses are not formed; that the proteid passes directly to deuteroproteose and thence into true peptone, etc.[3] Further, it is implied in the scheme of pepsin-proteolysis generally accepted by writers on this subject, that antialbumid undergoing gastric digestion is transformed directly into antideuteroalbumose without any intermediate formation of a primary proteose.[4] This, however, is not the case; certainly not where pure antialbumid is undergoing digestion. Thus, we have observed that in the digestion of antialbumid with both gastric juice and pancreatic juice, the albumoses formed and separated contained a large amount of protoalbumose; the neutral solution of the albumoses yielding a very decided precipitate on saturation with sodium chloride.

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[1] The trypsin solution was prepared by warming 40 grams of Kühne's dry ox pancreas in 400 c.c. 0.1 per cent salicylic acid solution for 24 hours, making the extract alkaline (0.25 per cent Na_2CO_3) and warming this at 40° C. for 48 hours in the presence of thymol. The extract was next dialyzed for some days from both alkaline and acid (acetic acid) solution, then evaporated to dryness at 40° C. and the residue taken up with a very little cold water. This solution, filtered, contains fairly pure trypsin.

[2] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1883, xix, p. 166.

[3] See NEUMEISTER's Lehrbuch d. physiol. Chem., 1897, p. 247.

[4] See NEUMEISTER: Zeitschr. f. Biologie, 1887, xxiii, p. 391.

The antipeptone (D) formed by the action of trypsin on pure antialbumid was found on analysis to have the following composition, when dried at 110° C.

I. 0.3003 gram substance gave 0.1812 gram $H_2O = 6.70$ per cent H and 0.5502 gram $CO_2 = 49.96$ per cent C.
II. 0.3330 gram substance gave 0.6075 gram $CO_2 = 49.75$ per cent C.
III. 0.2681 gram substance gave by the Kjeldahl method 0.03570 gram N = 13.32 per cent N.
IV. 0.2722 gram substance gave 0.03612 gram N = 13.27 per cent N.
V. 1.0382 grams substance gave by fusion with NaOH[1] and KNO_3 0.1203 gram $BaSO^4$ = 1.59 per cent S.
VI. 1.0665 grams substance gave 0.0228 gram ash = 2.14 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C . . .	51.05	50.82	....	....	....	50.93
H . . .	6.84	....	....	....	....	6.84
N . . .	....	....	13.61	13.56	....	13.58
S . . .	....	....	....	....	1.62	1.62
O . . .	....	....	....	....	....	27.03
						100.00

This is the only analysis, so far as we are aware, of an antipeptone prepared from pure antialbumid. Some years ago Kühne and Chittenden[2] analysed a preparation of so-called antipeptone prepared by the digestion of antialbumid with alkaline pancreatic juice, but at that time the proteoses had not been discovered and consequently the method of separation made use of did not effect the removal of the antialbumoses; hence, the product analyzed was without doubt a mixture of true antipeptone and antialbumoses. In considering the composition of this product it will be remembered that antialbumid is characterized by a comparatively high content of carbon and a low content of nitrogen (53.79 per cent C and 14.55 per cent N),[3] hence it is to be observed that in the formation of this peptone, as in all cases of peptone formation, the mother proteid loses an appreciable amount of carbon.[4] Somewhat noticeable in this product is the comparatively high content of sulphur, for peptones as a rule contain a very small amount of this element. There was no sulphur, however, present in the mercaptan form, and the ash contained no sulphate. Neither was there any evidence of the presence of ammonium sulphate. As to the relationship in composition between this antipeptone and the antipeptone resulting from the pancreatic digestion of a native proteid it will suffice here to refer the reader to previous papers on antipeptone.[1]

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[1] Pure sodium hydroxide made from the metal sodium and free from sulphur. The fusion was made in a silver crucible over an alcohol lamp.

[2] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1883, xix, p. 169.

[3] KÜHNE and CHITTENDEN: *loc. cit.*, p. 167.

[4] See CHITTENDEN: Digestive proteolysis, New Haven, 1895, p. 70.

Another product to be referred to under the head of anti bodies is *antialbumid* (*F*). This substance was prepared from well crystallized edestin,[2] a globulin obtained from hemp-seed, by heating the substance at 100° C. with 4 per cent sulphuric acid. The gelatinous antialbumid resulting by this process was purified by solution in 0.5 per cent sodium carbonate, etc., and then used for intravenous injection.

Hydrolysis and cleavage of pure gelatin by trypsin in neutral solution. — In this hydrolysis 200 grams of exceptionally pure gelatin prepared from the purified collagen of white fibrous connective tissue (tendons) by Dr. Van Name[3] were available. This material was dissolved in four litres of distilled water, a neutral solution of purified trypsin[4] added, together with sufficient thymol to prevent putrefaction, and the mixture warmed at 40° C. for four weeks. The object of this long-continued digestion was to insure the formation of as large an amount as possible of true gelatin-peptone, the substance usually described under this name being nothing more than a mixture of gelatoses. Further, digestion was carried on in a *neutral* fluid to obviate the necessity for removal from the resultant products of alkali salts; a process which entails great loss of substance. Careful examination of the digestive mixture at the end of the four weeks showed the complete absence of primary gelatoses. The solution was therefore concentrated and the deuterogelatose separated directly by saturation with ammonium sulphate under the conditions already referred to. The product was purified by dialysis, and eventually precipitated from a concentrated solution by strong alcohol, after which it was repeatedly boiled with alcohol for removal of any soluble extractives and then dried. This product constitutes *deuterogelatose* (*K*). The yield was 113 grams.

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[1] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1886, xxii, p. 423; CHITTENDEN: Studies in physiological Chemistry, Yale University, 1889, iii, p. 100; CHITTENDEN and GOODWIN: Journal of physiology, 1891, xii, p. 34; BALKE: Zeitschr. f. physiol. Chem., 1896, xxii, p. 248, etc.

[2] CHITTENDEN and MENDEL: Journal of physiology, 1894, xvii, p. 48.

[3] VAN NAME: Journal of experimental medicine, 1897, ii, p. 117.

[4] See foot-note (p. 174).

The ammonium sulphate-saturated fluid containing the true gelatin-peptone formed by trypsin was freed from the excess of ammonium salt by crystallization, the residue removed by treatment with barium hydroxide and barium carbonate as described in the preparation of hemipeptone, and the true peptone precipitated by alcohol. After thorough and repeated extraction with boiling alcohol the peptone was dried. *Gelatin-peptone* (*L*). It weighed 17 grams. The relative yield of deuterogelatose and true peptone in this long-continued hydrolysis with an active solution of trypsin constitutes striking evidence of the relatively slow production of true gelatin-peptone in trypsin-proteolysis.

The deuterogelatose in aqueous solution gave no precipitate whatever on saturation of the fluid with sodium chloride, neither did any precipitate result on addition of acetic acid to the salt-saturated fluid. Consequently, it must be considered as entirely free from protogelatose.[1] Tested with potassium hydroxide and plumbic acetate for loosely combined sulphur, both peptone and gelatose gave negative results. Negative results were likewise obtained with Millon's reagent and the xanthoproteic test. With the biuret test, strong solutions of both gelatose and peptone yielded a bright pink color. The composition of these two products is shown by the following results.

Analysis of Deuterogelatose (*K*).[2]

I. 0.2811 gram substance gave 0.1705 gram H_2O = 6.74 per cent H and 0.5201 gram CO_2 = 50.46 per cent C.

II. 0.2895 gram substance gave 0.1769 gram H_2O = 6.79 per cent H and 0.5356 gram CO_2 = 50.45 per cent C.

III. 0.3195 gram substance gave by the Kjeldahl method 0.05556 gram N = 17.39 per cent N.

IV. 0.2952 gram substance gave 0.05134 gram N = 17.39 per cent N.

V. 0.5166 gram substance gave by fusion with NaOH and KNO_3 0.0126 gram $BaSO_4$ = 0.33 per cent S.

VI. 0.3695 gram substance gave 0.0030 gram ash = 0.81 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C . . .	50.87	50.86	....	....	....	50.87
H . . .	6.79	6.84	....	....	....	6.81
N . . .	....	....	17.53	17.53	....	17.53
S . . .	....	....	....	....	0.34	0.34
O . . .	....	....	....	....	....	24.45
						100.00

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[1] See CHITTENDEN and SOLLEY: Journal of physiology, 1891, xii, p. 33.

[2] Dried at 105° C. until of constant weight.

Analysis of Gelatin-peptone (L).

I. 0.2984 gram substance gave 0.1797 gram H_2O = 6.69 per cent H and 0.5168 gram CO_2 = 47.23 per cent C.

II. 0.2134 gram substance gave 0.1286 gram H_2O = 6.69 per cent H and 0.3709 gram CO_2 = 47.40 per cent C.

III. 0.2869 gram substance gave by the Kjeldahl method 0.04827 gram N = 16.82 per cent N.

VI. 0.2168 gram substance gave 0.03661 gram N = 16.88 per cent N.

V. 0.5017 gram substance gave by fusion with NaOH and KNO_3 0.0119 gram $BaSO_4$ = 0.32 per cent S.

VI. 0.5376 gram substance gave 0.0114 gram ash = 2.12 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C. . . .	48.25	48.42	....	....	....	48.33
H . . .	6.83	6.83	....	....	....	6.83
N. . . .	....	....	17.18	17.24	....	17.21
S	....	....	....	....	0.33	0.33
O. . . .	....	....	....	....	....	27.30
						100.00

Comparing these results with the composition of the pure gelatin from which the gelatose and peptone were derived we have the following figures:

	Gelatin[1]	*Deuterogelatose*	*Gelatin-peptone*
C	50.11	50.87	48.33
H	6.56	6.81	6.83
N	17.81	17.53	17.21
S	0.25	0.34	0.33
O	25.24	24.45	27.30

Thus, again we have a forcible illustration of the fact that in both pepsin- and trypsin-proteolysis the formation of true peptone is associated with a marked diminution in the content of carbon.[2] The composition of the deuterogelatose, on the other hand, is seen to be essentially identical with that of the gelatin from which it was derived, thus agreeing with the observations previously recorded by Chittenden and Solley[3] on the composition of the gelatoses formed by gastric and pancreatic digestion.

The protogelatose (protogelatose *J*) tested intravenously was a specimen prepared by pepsin-proteolysis of a purified commercial gelatin,[4] and had the following composition:

C 49.98, H 6.78, N 17.86, S 0.52, O 24.86, Ash 1.98.

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[1] VAN NAME: *loc. cit.*, p. 124.

[2] CHITTENDEN: Digestive proteolysis, New Haven, 1895, p. 71.

[3] CHITTENDEN and SOLLEY: Journal of physiology, 1891, xii, p. 23.

[4] CHITTENDEN and SOLLEY: *loc. cit.*, p. 28.

ADDENDUM.

Since this work was completed two exceedingly interesting and important papers on antipeptone have appeared from Kossel's laboratory,[1] which have a direct bearing upon the purity of antipeptone formed from blood-fibrin by digestion with an alkaline pancreatic extract. Kutscher's results tend to show that ordinary antipeptone formed from blood-fibrin by pancreatic digestion and purified by the methods ordinarily made use of contains a large proportion of impurities composed in great part of the nitrogenous bases arginin, histidin, and a new body as yet undescribed, together with aspartic acid and some leucin and tyrosin. Assuming this to be true of all antipeptones formed from native proteids by direct digestion with pancreatic juice, it follows that the products of this kind hitherto tested physiologically are open to the suspicion of being unsuitable for experiments of this nature. How far the same criticism will apply to the antipeptone employed by us in the present experiments is uncertain. It would seem, however, that the antipeptone prepared from antialbumid might perhaps be free from this objection or at least contain a far smaller amount of impurity, since in the hydrolysis and cleavage of egg-albumin in the preparation of antialbumid the more easily decomposable half of the proteid molecule would be at once eliminated. Consequently in forming antipeptone from pure antialbumid by trypsin-proteolysis it is quite possible that the resultant peptone is free from the objectionable features of ordinary antipeptone owing to the nature of the mother substance. If such is the case our present experiments possess a double value. In partial justification of the belief that antipeptone prepared from antialbumid is less liable to contain the impurities referred to is the fact that the well known bodies leucin and tyrosin, so common among the products formed in the breaking down of a native proteid with trypsin and usually associated with the nitrogenous bases, are never found in the proteolysis of pure antialbumid. Further, in the original analyses of antipeptone made by Kühne and Chittenden,[2] it was found that purification by phosphotungstic acid tended to raise decidedly the content of nitrogen; a fact which Kutscher explains by assuming the more complete precipitation of the nitrogenous bases by this reagent. Thus, in a preparation of fibrin-antipeptone containing 16.58 per cent of nitrogen, precipitation by phospho-tungstic acid resulted in raising the content of nitrogen in the substance to 18.28 per cent.[1] In other words, this method of purification led, according to Kutscher, to an increase in the proportion of impurities. This being so, the higher content of nitrogen in an antipeptone is to be looked upon with suspicion. Accepting the truth of this statement, the extremely low content of nitrogen in the antipeptone from antialbumid (13.58 per cent) may perhaps be taken as additional evidence of the freedom of the peptone from the objectionable nitrogenous bases. It is not to be understood from this hypothesis that the anti bodies, antialbumid or antipeptone, will not yield nitrogenous bases on decomposition. The real distinction between a hemi and anti body is based upon the behavior of the substance toward trypsin. As we have already pointed out, antialbumid which will not yield tyrosin on treatment with trypsin, will yield considerable tyrosin on decomposition with a strong mineral acid at 100° C. So likewise, these anti bodies may yield a large proportion of the nitrogenous bases on decomposition with a boiling mineral acid while wholly resistant to the action of trypsin.

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[1] KUTSCHER: Zeitschr. f. physiol. Chem., 1898, xxv, p. 195; KUTSCHER: *ibid*, 1898, xxvi, p. 110.

[2] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1886, xxii, p. 435.

In this connection it has seemed to us important to ascertain how large a yield of nitrogenous bases can be obtained from antialbumid by decomposition with a mineral acid, and also to compare the results with those obtainable by a like decomposition of a hemi body. For this purpose, 10 grams each of antialbumid, hemialbumoses, and hemipeptone were boiled for 96 hours with 40 c.c. of 20 per cent hydrochloric acid with addition of a little stannous chloride. The solutions were then diluted with water, the tin removed by hydrogen sulphide, and the filtrates evaporated to syrupy consistency. The residues were then dissolved in water, and each made up to 250 c.c. Measured portions of these solutions were used for the determination of total nitrogen, nitrogen as ammonia, and basic nitrogen precipitated by phospho-tungstic acid, following the methods used by E. Schulze.[2] Following are the results obtained:

Antialbumid (A)

Total nitrogen in the fluid	1.2745 grams.
Nitrogen in phospho-tungstic acid precipitate	0.2782 "
Nitrogen in the form of ammonia	0.0585 "
Basic nitrogen, organic	0.2197 "

17.2 per cent of total nitrogen in the form of bases precipitable by phospho-tungstic acid.

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[1] KÜHNE and CHITTENDEN: *loc. cit.*, p. 452.

[2] SCHULZE, E.: Zeitschr. f. physiol. Chem., 1898, xxv, p. 360.

Hemialbumoses (*G*)

Total nitrogen in the fluid	1.3917 grams.
Nitrogen in phospho-tungstic acid precipitate	0.4687 "
Nitrogen in the form of ammonia	0.0805 "
Basic nitrogen, organic	0.3882 "

27.9 per cent of total nitrogen in the form of bases precipitable by phospho-tungstic acid.

Hemipeptone (*I*)

Total nitrogen in the fluid	1.3770 grams.
Nitrogen in phospho-tungstic acid precipitate	0.5492 "
Nitrogen in the form of ammonia	0.2637 "
Basic nitrogen, organic	0.2855 "

20.7 per cent of total nitrogen in the form of bases precipitable by phospho-tungstic acid.

From these results we see that antialbumid differs only quantitatively from the hemi bodies in yielding nitrogenous organic bases on decomposition with a mineral acid; a fact which affords additional evidence that the difference between the so-called hemi and anti bodies is not due to the lack of specific groups or radicles in the anti body, but rather to some difference in the arrangement of the groups or the state of combination by which the latter body is rendered resistant to the enzyme trypsin. Particularly noteworthy is the large proportion of nitrogen split off from hemipeptone in the form of ammonia, over 19 per cent of the total nitrogen appearing in that form.

In order to ascertain how far the possible presence of these "hexone" bases in antipeptone may affect the physiological action of the latter substance, an experiment was tried with a dog of 2.5 kilos body-weight in which 25 c.c. of a solution containing 0.73 gram of the mixed bases (from the phospho-tungstic acid precipitate) in the form of acetates were injected intravenously. The substance injected contained 15.5 per cent of nitrogen when dry. As a result it was found that the clotting time of the blood was not noticeably affected, while arterial pressure quickly fell from 135 mm. to 50 mm. Hg., but returned to the normal within three minutes.

ON THE COURSE OF IMPULSES TO AND FROM THE CAT'S BLADDER.

BY COLIN C. STEWART, PH.D.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

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THE experimental study here reported was undertaken in the Laboratory of Physiology of the Harvard Medical School, under the direction of Prof. W. T. Porter. To him I owe much, both for useful suggestions and for guidance throughout the work. The original intention was to determine, if possible, the exact nature of the mechanism for the transmission of crossed impulses in the region of the reflex bladder centre, as has already been done[43] for the impulses reaching the diaphragm through the phrenic nerves. So much routine work has developed, however, that the present paper is published with no reference to the original theme. The course of the impulses, the demonstration of the existence of physiologically continuous paths, and the definition of their limits, are all that have been attempted.

THE PERIPHERAL MOTOR NERVES.

As a preliminary to the experiments of the present research the course of the peripheral nerves to the bladder was studied both by dissection and by experiment, in several cats. In these operations, and in all others which were performed, the animals were fully anæsthetized with ether. Nothing was found at variance with the general results already described in the earlier literature[1] of the subject.

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[1] For the topography of the motor nerves of the bladder see: Valentin[1]; Budge[5] (p. 118); Gianuzzi[6]; Budge[7] (p. 14)[8 17]; Sokownin[20 21]; Nussbaum[22 24]; Mosso and Pellacani[26] (p. 300), [27] (p. 43); Nawrocki and Skabitschewsky[30] (pp.

Motor fibres were found in the II and III sacral roots, and in the nervus erigens, which sends large strands to the hypogastric plexus. The question as to whether fibres from the I or IV sacral root are also carried toward the bladder by the nervus erigens was necessarily disregarded, for in none of the experiments which were undertaken was it possible first to explore the spinal nerves for variations, although such variations are of frequent occurrence. To avoid error, therefore, the sacral motor supply was regarded as a unit, and when it was to be extirpated at its exit from the cord all the sacral nerves were cut.

The fibres of the sympathetic group are carried regularly in the III, IV, and V lumbar roots, but the strands leading the impulses from the sympathetic chain to the inferior mesenteric ganglion vary much in position. They are usually four in number on each side, but sometimes three and sometimes five were observed; of fifteen cats eight had four, four had three, and three had five. The cases in which each strand proceeds separately to the ganglion are rare; usually two or three unite at some point in their course, and sometimes a plexus is formed. Their origin from the sympathetic chain is also subject to some variation, but they arise always in the region of the III, IV, and V lumbar segments. From the inferior mesenteric ganglion the hypogastric nerves convey the impulses to the hypogastric plexus, but accessory hypogastrics, as Langley and Anderson[41] (p. 413) have mentioned, are of frequent occurrence. Whenever such were present they were treated as parts of the hypogastric nerve itself.

The accompanying drawing is made from the dissection of a special case, and the nerves of the right side only are plotted. Four strands run from the lumbar sympathetic chain to the inferior mesenteric ganglion. From the ganglion arises the right hypogastric nerve, which in its course gives off an accessory hypogastric. Three branches from the nervus erigens unite with these two nerves to form the pelvic, or hypogastric plexus, and from the plexus four terminal branches run to the bladder. The number of terminal branches is more usually three, as in the dog (Griffiths[44], p. 64).

338-339)[31]; Langley[32] (pp. xxxiii and xxiv); Sherrington[33] (pp. 640, 678); Langley[36] (p. 237); Langley and Anderson[41] (p. 412); Griffiths[44] (pp. 62-67); François-Franck[38] [46]; Langley and Anderson[47] (p. 79),[48] (pp. 76, 77, 78, 81, and 84), and[49] (pp. 374-379); and Courtade and Guyon[50] (p. 623). (The number after each name refers to the list of references at the end of the paper.)

The larger ganglia of the hypogastric plexus and of the vesical plexus on the surface of the bladder are also shown.

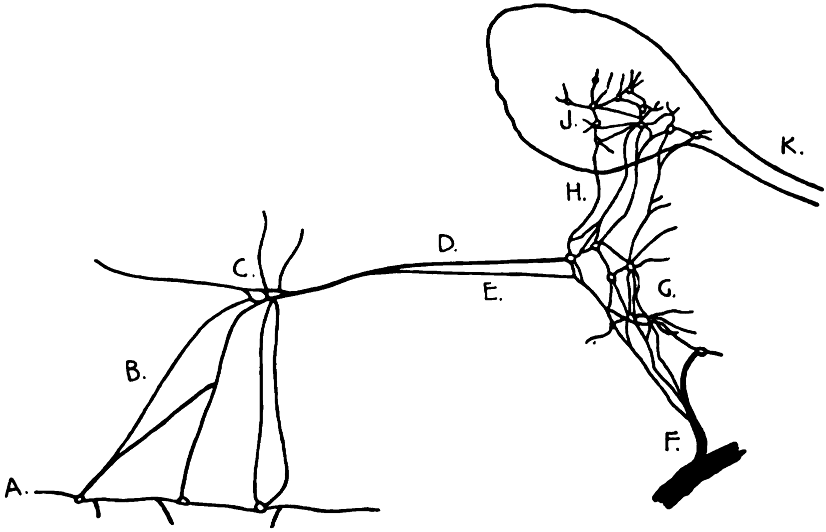

FIGURE 1. The vesico-motor nerves of the cat, on the right side, showing: A, the sympathetic chain, B, the rami to C, the inferior mesenteric ganglion, D, the hypogastric, and E, the accessory hypogastric nerve, F, the nervus erigens, G, the hypogastric plexus, H, the terminal nerves, J, the vesical plexus on the surface of the bladder, and K, the urethra.

It has been found impossible to demonstrate the presence in the sympathetic chain of vesico-motor fibres other than those carried in the paths described, that is, through the hypogastric nerves. Such fibres have been described by Gianuzzi[6] (p. 23), and Nussbaum[24]; while Sokownin,[21] and Nawrocki and Skabitchewsky[30] (p. 340, and Exp. III, p. 343) have failed to demonstrate their presence. Langley and Anderson[48] (p. 76) have obtained positive results in certain cases only. Though in some cases nerves which correspond to those described by Langley and Anderson (op. cit.) have been seen, they could not be proved to carry fibres having any control over movements of the bladder. Section of the hypogastric nerves left only the sacral paths open and subsequent section of the sacral spinal nerves destroyed all possibility of producing bladder contractions by stimulating the cord. This was shown several times also incidentally in experiments undertaken for other purposes.

THE SENSORY NERVES OF THE BLADDER, AND THE REFLEXES.[1]

While the motor nerves may be explored by methods of direct stimulation with comparative ease, the demonstration of sensory nerves for the bladder is a matter of extreme difficulty. The methods are two in number. First, a nerve leading toward the bladder, or its central cut end, is stimulated while the animal is watched for respiratory or general body movements giving evidence of sensation. Second, the central cut end of a nerve suspected to contain sensory bladder fibres may be stimulated while the bladder is watched for contractions of reflex origin. Both methods are open to serious objections. Even if stimulation of the central cut end of a nerve trunk leading toward the bladder give evidence of having produced sensation, there is nothing to show that the sensory fibres stimulated belong to the bladder, unless the point of stimulation be between the bladder and the hypogastric plexus. Centrally of that point all motor nerves to the bladder are accompanied by similar nerves for the lower part of the intestine and for the genitals, and doubtless sensory nerves are combined in the same way. Again, it has been shown repeatedly that stimulation of almost any sensory nerve of the body will cause a reflex contraction of the bladder. Therefore such a reflex contraction produced by stimulating the central cut end of any nerve known to contain fibres for organs other than the bladder, cannot be taken as an index.

It has been stated (Sokownin[20]) that sensory nerves other than those belonging properly to the bladder have their reflex centre in the brain, while the bladder's own nerves produce reflexes from the vesical centre in the cord. If this were so, we should be provided with an easy means of distinguishing the true sensory nerves of the bladder; but this view is untenable. A reflex contraction of the bladder may be obtained by stimulating the central cut end of the sciatic nerve even immediately after section of the cervical or thoracic cord, as Mosso and Pellacani[26] (p. 292) pointed out. Nor is an injection of strychnine necessary for the demonstration of the reflex. It is so constant that it may be used to test the condition of the spinal vesical centre, and as a means of determining the upper level of the centre.

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[1] The following references are of importance: Longet[4] (p. 614); Budge[7]; Paul Bert[13]; Gianuzzi[14,15]; Budge[17]; Goltz[18]; Sokownin[20,21]; Nussbaum[22,24]; Mosso and Pellacani[26,27]; Nawrocki and Skabitschewsky[31]; Langley[32]; Langley and Anderson[39,40,43]; Griffiths[44]; and Courtade and Guyon[50].

It may be demonstrated, however, that the sacral nerves to the bladder contain sensory fibres. If the hypogastric nerves be cut to interrupt the connection with the lumbar cord, and if the peripheral bladder nerves be severed close to the bladder on one side, careful stimulation of their central divided ends will produce a reflex contraction. The path of this reflex is through the sacral nerves and through the cord, for if the sacral nerves of the opposite side be then cut, or if the lower lumbar or upper sacral cord be interrupted or destroyed, the reflex fails. If the first section of the experiment were made at any point in the course of the sacral nerves central to the hypogastric plexus, the reflex contraction produced by stimulating the central cut ends would be valueless. Doubtless the sensory nerves of the bladder are accompanied from that point by afferent nerves from other organs.

If the sacral nerves, thus shown to contain sensory nerves for the bladder, be cut on both sides, stimulation of their central cut ends produces no effect upon the bladder even though the hypogastric nerves are left intact. The hypogastric nerves do not carry motor impulses aroused by stimulating any of the afferent fibres contained in the sacral nerves. The sacral nerves are therefore both afferent and efferent for the reflex described.

It may be shown in a similar way that the hypogastric nerves contain afferent bladder fibres. If the terminal nerves to the bladder be cut on one side, and if the sacral supply to the hypogastric plexus be severed on both sides, stimulation of the central divided end of the peripheral nerves gives a well marked contraction of the bladder. This may be produced in either one of two ways. It may be a reflex from the inferior mesenteric ganglion, or it may be a reflex from the cord.

If now the connections of the inferior mesenteric ganglion with the cord be severed, stimulation of the central end of the divided peripheral nerves produces the reflex contraction as before. Such a reflex from the inferior mesenteric ganglion has already been demonstrated many times[1] by stimulating the central cut end of one hypogastric nerve, with the inferior mesenteric ganglion isolated. But the reflex obtained in this way might be mediated by afferent nerves from other organs supplied by the hypogastric nerves, and cannot be taken as demonstrating the presence of sensory fibres for the bladder in the hypogastrics. The point of stimulation must be peripheral to the hypogastric plexus. That the reflex centre is not in the cord may be shown in an animal in which the whole nerve supply to the bladder is intact. Stimulation of the central cut ends of the branches which unite the sympathetic chain with the inferior mesenteric ganglion produces no contraction of the bladder.

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[1] See Sokownin[20], Nussbaum[22-24], Nawrocki and Skabitschewsky[81] (p. 156), Langley and Anderson[39] and [41] (p. 415).

Sensory fibres are present, then, in the sacral supply to the bladder, and in the hypogastric nerves. Failure to demonstrate them between the inferior mesenteric ganglion and the cord, agrees well with three previous observations: Mosso and Pellacani[26] (p. 302) found that the bladder lost neither in sensation nor in motion after extirpation of the sympathetic rami to the ganglion; Langley and Anderson[40] (p. 188) have pointed out that the relative number of afferent to efferent fibres is smaller in the sympathetic rami than in the hypogastrics; and Griffiths[44] (p. 76) has found the effect of stimulating the central cut end of the hypogastric nerve upon the central nervous system to be wanting in experiments where similar stimulation of the sacral nerves has produced profound disturbances.

There is still another reflex produced by what we know to be sensory bladder nerves. Griffiths[44] (p. 78) has described what he considers probably a genuine reflex from the cells in the hypogastric plexus. On isolating this plexus and stimulating the central cut end of one of the three terminal branches to the bladder a contraction results. This has been repeatedly confirmed in the present research. The reflex may be obtained with electrical stimuli, with the mechanical stimulus of pinching, and with stimulation by means of a hot wire.

With regard to the reflexes produced by stimulation of sensory nerves other than those of the bladder, many observations have already been made.[1] Stimulation of the central cut end of the divided sciatic nerve produces a strong contraction of the bladder. If the sacral supply to the hypogastric plexus be cut on both sides the reflex fails altogether. None of the motor impulses from the reflex centre appears to be carried by the lumbar sympathetic nerves. Stimulation of the central cut end of the vagus in the neck was found ineffectual in all cases.[2]

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[1] Paul Bert[18] (p. 650); Sokownin[20, 21]; Nussbaum[22, 24]; Mosso and Pellacani[26] (p. 291); Nawrocki and Skabitschewsky[31] (p 155).

[2] For the literature upon this point the reader is referred to: Oehl[9] (p. 341),

THE POSITION OF THE REFLEX CENTRE IN THE CORD.

In determining the position of the centre in the cord from which reflex bladder contractions may be produced, no attempt was made to mark the lower level of the centre, no suitable method being devised. Its upper limit was determined by a method of sections, using the reflex obtained from the sciatic nerve as the test. The animal used was anæsthetized with ether, and a tube was placed in the trachea. The abdomen was opened in the linea alba, and in most cases the symphysis pubis was divided. A catheter was then passed up the urethra well into the bladder, and tied in place. By means of the catheter the bladder was partially emptied, and filled again with warm sodium chloride solution (0.9 per cent), through a side branch in a tube leading to a horizontal manometer. A shield, solid at the sides but made of wire netting at the upper end, was placed over the bladder and held in position by the body walls, which were drawn together and clamped. The animal was then turned back up, and the cord and sciatic nerves were exposed, the cord being kept under a constant stream of normal saline solution at body-temperature.

The sciatic nerves were then cut, and the central end of one of them was stimulated by means of an induced current strong enough to be distinctly felt on the tongue. An outward movement of the fluid in the manometer invariably followed. The cord was now cut across with a sharp knife and the reflexes were tested again. If the reflexes still persisted a second and lower cut was made. Sections above the level of the second lumbar root were always without effect, but any transverse section below the origin of the second lumbar root abolished the reflex.

In one series of these experiments the animal was anæsthetized, and the cord cut, from five to twenty-four hours before the final operation, the cat being kept under morphia in the intervening time. The purpose of this was to avoid any possible effect of shock upon the reaction of the reflex centre to stimulation through the sciatics. As before, the reflexes persisted after any section above the origin of the second lumbar root, and were abolished by cutting the cord anywhere below that point.

and [11], and Nussbaum,[22] who have reported positive results, and to Budge[7] (p. 11), Kehrer,[10] Paul Bert[18] (p. 651), Sokownin,[20] [21] Nussbaum,[24] and Nawrocki and Skabitschewsky[81] (p. 255), for negative results.

The upper limit of the reflex bladder centre is then somewhere near the lower level of the origin of the second lumbar root. This does not agree with some of the results which have been obtained by former investigators. Budge's centre[5] (p. 118),[7] (p. 13), opposite the IV lumbar vertebra in the rabbit, was the point in the cord where irritability was greatest. But in many of the experiments of the present research it has been noticed, in work upon the lumbar cord, that after the cord ceases to conduct impulses at all, if the electrodes are brought in the region of the IV and V lumbar root origins, strong contractions of the bladder result. This has occurred even when the cord was destroyed by repeated longitudinal sections, so that the obvious explanation of Budge's results is that motor fibres were stimulated, not the cells in the centre. Masius[12] (p. 502), Gianuzzi,[14 15] Kupressow,[16] and Ott[23] (p. 61), place the centre for tonus of the vesical sphincter at about the level of the V lumbar root; but the whole bladder centre is undoubtedly of much larger extent in the cord. Gianuzzi[6] (p. 28) demonstrated two centres, the upper one opposite the III lumbar root; Sokownin,[20 21] places the centre opposite the IV lumbar; Nussbaum[22 24] describes the region of the III and IV lumbar roots; and Nawrocki and Skabitschewsky[31] (p. 155) give the II and V lumbar roots as the limits of the centre. These latter determinations all agree more or less closely with the one made in the present research.

THE EFFECTS OF STIMULATING THE PERIPHERAL NERVES.

The musculature of the bladder is of such a nature that the bladder may be considered as belonging to that group of the viscera in which the muscles are arranged in two layers, — longitudinal and circular. The longitudinal muscles, the detrusors, are more powerful in the wall of the viscus, while the circular muscles are strongest in the sphincter in the neck; thus a physiological separation might be sought for. Longet[4] (p. 614) indeed describes the sympathetic nerves as controlling the body of the bladder, while the sacral nerves control the sphincter. Von Zeissl[34 35] finds that the bladder conforms to von Basch's law of crossed innervation. The nervus erigens is described as motor for the body and inhibitory for the sphincter, while the hypogastric nerve closes the sphincter and inhibits the muscles of the body of the bladder. Courtade and Guyon[50] (pp. 625–626) also have given the hypogastrics and the sacral nerves as motor for the neck and the longitudinal muscles respectively. But against these observations are those of Gianuzzi[8] (p. 24), Mosso and Pellacani[26] (p. 300),[27] (p. 43), Langley,[32] Sherrington[33] (p. 679), Griffiths[44] (p. 73), and Langley and Anderson,[48] who have found, in general, that the effect of stimulating the hypogastrics is different only in degree from that produced by stimulating the more powerfully motor sacral nerves.

Stimulation of the bladder, whether it be reflex or direct, and whether it be through the sacral or the sympathetic supply, produces a contraction which involves all parts of the musculature. Nothing has been observed which lends any support to the view that the innervation is crossed. There is, however, a difference in the effect of stimulating the hypogastric nerves, noted first by Langley[32], and later by Langley and Anderson[41 48] (p. 75), and Griffiths[44] (p. 74), according to whom stimulation often produces a dilatation, usually preceded by a short preliminary contraction.

Stimulation of the sympathetic supply at any point in its course produces a rise in the internal pressure of the bladder, followed usually by a fall of much greater extent than the original rise. The ratio may be as great as three to one. This is produced whether the lumbar spinal nerves, the rami to the inferior mesenteric ganglion, or the hypogastric nerves be stimulated, though more often one or two of the rami have an effect upon the bladder which is greater than that produced by the others. Several attempts were made to separate the fibres, stimulation of which produces the rise, from those which produce the fall, but they were always found to occur together anatomically. The injection of nicotine has in some instances seemed to affect the proportion between the rise and the fall, but no results of definite value were obtained, nor were any results obtained by the use of induction shocks, made to follow each other at varying intervals by using Ewald's interruptor.

Griffiths[44] (p. 74) found that stimulation of the hypogastric during the progress of contraction induced by stimulation of the pelvic splanchnic produced no result, an observation confirmed by Langley and Anderson[48] (p. 75), who found no satisfactory evidence of the presence in the hypogastrics of inhibitory fibres, nor any obvious inhibitory effect on the bladder when it had become contracted in consequence of exposure to the air. But very frequently in the course of the experiments here described it has been observed that when the bladder was insufficiently dilated to make the manometric variations apparent, stimulation of the hypogastric nerves was effectual in producing a dilatation. Often warm normal saline solution was poured over the exposed surface of the bladder to produce the same effect, but the method of stimulation was nevertheless in common use.

Stimulation of the peripheral cut ends of any or all of the sympathetic rami to the inferior mesenteric ganglion on either side produces the same effect as stimulation of the nerves in their continuity. So also does stimulation of the inferior mesenteric ganglion with its spinal connections wholly or in part severed. If, however, the spinal rami, or the ganglion, be stimulated after section of one hypogastric nerve, then the bladder contracts only on the side with the intact hypogastric nerve. Similarly if the peripheral cut end of the divided hypogastric nerve be stimulated, contraction results only on the stimulated side, as noted by Langley and Anderson[41] (p. 423), and Griffiths[44] (p. 73). And again, the reflex contraction of the bladder produced by stimulating the central cut end of one hypogastric nerve involves only the side of the bladder supplied by the intact nerve.

The effect upon the internal pressure of the bladder of stimulating only one hypogastric nerve is not, however, the same as that produced by stimulation through both. In the latter case, as has already been stated, the manometer shows first a rise and then a fall. Stimulation through but one hypogastric causes the usual contraction of the corresponding half, and passive bulging of the other. When the stimulated side begins to relax in the typical way, the passive side contracts once more, and thus no great fall of internal pressure is recorded by the manometer.

In the case of the sacral nerve supply to the bladder stimulation of the sacral roots, of the nervus erigens, of the hypogastric plexus, or of the peripheral fibres, causes a strong contraction, followed by a slow return to the original form. Stimulation of the nerves in their continuity affects both sides of the bladder alike; but if the sacral supply be cut, and the peripheral end be stimulated, then contraction of only the corresponding half results. If the hypogastric plexus or the peripheral branches from the plexus to the bladder be cut in this way it is necessary to sever also the hypogastric nerve to prevent a reflex from the inferior mesenteric ganglion to the opposite half of the bladder. If, also, the central cut end of the divided sacral supply be stimulated, the resulting reflex contraction involves only the half of the bladder with the intact nerves. Gianuzzi[6] (p. 24), Sherrington[33] (p. 683), and Griffiths[44] (p. 73), have described the effect of stimulation as unilateral; Langley and Anderson[48] (p. 80) do not agree with the earlier observations.

Relatively much stronger stimuli are required for the sympathetic nerves than for those of the sacral group. Even the strongest electrical stimulus applied to the sympathetic nerves will never produce so quick or so forcible a contraction as that commonly obtained from the sacral fibres. Mechanical stimulation, whether by pinching, ligating, cutting, or stretching the nerves, is effectual almost always; but the effect produced through the sacral nerves is always the greater.

Very frequently when the bladder is contracted, stimulation, even of the sacral nerve supply, produces relaxation, when the same stimulus would cause contraction if the bladder were at rest.

It has been observed repeatedly, especially on stimulating the sacral nerves, that the effect of a single stimulation is not a single contraction but a regular rhythmical series of contractions. Such a series is composed of from four to six oscillations, gradually diminishing in intensity.

There are differences in the persistence of irritability in the different regions of the peripheral nerve supply of the bladder, after the stoppage of the circulation. Nawrocki and Skabitschewsky[30] (p. 342) found irritability persisting in the hypogastrics for about an hour, in the sympathetic rami a shorter time. Usually the sympathetic rami cease to respond to stimulation almost immediately after the heart ceases to beat. In the hypogastrics irritability disappears after a very few minutes; but the bladder will still respond to stimulation of the nerves of its sacral supply sometimes for more than an hour. Since the differences depend doubtless on the fact that sympathetic cells die earlier than their fibres, as Langendorff[29] (p. 131) has pointed out, his method of determining the positions of the cells seems especially applicable to the study of the innervation of the bladder.

A constant possibility of error in the study of the effects of stimulating either sensory or motor bladder nerves is to be found in the rhythmic contractions normally present. These have been mentioned by Mosso and Pellacani[25] (p. 103), Ashdown[28] (p. 316), Sherrington[33] (p. 682), who showed that the rhythmic action arises in the wall of the bladder itself, and by Langley and Anderson[41] (p. 416), and Griffiths[45] (p. 254), who describe them as easily distinguished from those produced by stimulation. The rhythmical variations in pressure which have been observed in the cat's bladder during the present research were not of such a nature as to seriously interfere with the experiments. In only one case was it necessary to abandon the experiment. Usually the amplitude of the variations is much slighter than that produced by stimulating any of the nerves. Sometimes the spontaneous contractions follow each other irregularly, but more often the rhythm is a regular one with intervals of from five to fifteen seconds. Ashdown[28] (p. 317) found the interval to be much longer. The opening of the body cavity had no constant effect either to increase or to abolish the rhythmical contractions.

THE COURSE OF IMPULSES IN THE CORD AND IN THE PERIPHERAL NERVES.

Budge[2] (p. 159), [17] (p. 310), and Valentin[3] (p. 327) showed that contractions of the bladder may be produced by stimulating the exposed cord. Nussbaum,[24] Mosso and Pellacani,[26, 27] Nawrocki and Skabitschewsky,[30] and Sherrington[12] (p. 71) have confirmed the original observations. Various observers have demonstrated the same effect upon stimulation of higher parts of the central nervous system, but the consideration of such results does not properly form a part of the present paper.

Bladder contractions may be produced by stimulating the cord at any point. The effect with both sets of peripheral motor nerves intact is the same as the effect produced by stimulating the nerves of the sacral group. If however the sacral nerves be excluded by section, then the effect is the same as that produced by stimulating the nerves of the lumbar group. Stimulation of the cord often causes an already contracted bladder to relax.

If the sympathetic rami to the inferior mesenteric ganglion be cut on one side, and if the sacral nerves be cut on the same side, stimulation of the cord still produces contraction through the uninjured nerves. If then a hemisection of the cord be made on the opposite side in the lower dorsal region, stimulation above the hemisection is still effective. The impulses are thus shown to cross somewhere in the cord below the level of the hemisection. It may be shown in the same way, by cutting the nerves and making the hemisection both on the same side, that the impulses which come down one side of the cord are able to produce contraction through the nerves of the same side.

Similarly, by excluding the nerves of each group separately, it can be shown that impulses coming down one side of the cord are distributed to the sympathetic nerves of both sides, and to the sacral nerves of both sides. And it has also been shown that the reflex from the sciatic nerve crosses the cord and is effective upon the same side.

To determine the upper and lower limits of the crossing of impulses in the cord it is necessary only to split the cord in the median line until the crossing is stopped. The following protocol will give an example of such an experiment.

Cat XLIII, May 19, 1898. The animal was etherized and placed upon the operating board back down. A cannula was placed in the trachea, the abdomen opened in the median line, the symphysis pubis cut, and a catheter passed up the urethra, well into the bladder, and tied in position. The bladder was then filled with normal saline solution, warmed to body-temperature, and the catheter was connected with the tube of a horizontal manometer.

Both hypogastrics were then cut, and also the sacral nerves to the hypogastric plexus on the left side. The bladder was shielded by a case, made partly of solid metal, partly of coarse wire netting, and the abdominal walls were drawn together and clamped. The animal was then turned over and the lower dorsal and the whole lumbar cord was exposed by removing the arches of the vertebræ. The cord was kept in a pool of normal saline solution, at body-temperature, from the time of the first exposure to the end of the experiment.

A right lateral hemisection of the cord was made at the XII dorsal root. Stimulation well above the hemisection by means of an induced current so weak as to be imperceptible on the tip of the tongue, resulted in a contraction of the bladder. Beginning at the hemisection (see Fig. 2) the cord was then split down the middle line one centimetre at a time, and after each new cut the effect of stimulating the dorsal cord was tested. In Figure 2 the black line indicating the complete section of the cord in the median line is broken to indicate the points at which each successive section joined the preceding one. When the cord was split to a point just below the lowest point of origin of the V lumbar root, stimulation was no longer effective. The electrodes were then placed on the right side of the cord immediately below the hemisection, and a well marked contraction of the bladder resulted.

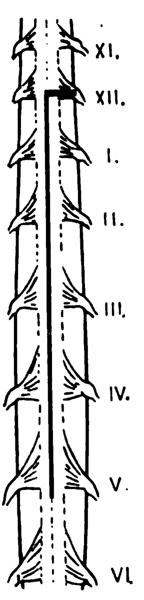

FIGURE 2. Diagram showing method of determining the lower level of the crossing of impulses in the cord.

In this way it was shown, in several experiments, that the lower limit of the crossing of impulses carried by the sacral roots is the lowest point of origin of the strands which make up the V lumbar root. In a series of experiments of a similar nature it was shown that the same point marks the lower limit of crossing of the impulses carried in the lumbar nerves, and of the impulses aroused reflexly by stimulating the central cut end of the divided sciatic. Each such experiment was at the same time used to prove that impulses also pass to the bladder uncrossed.

To determine the upper limit of the crossing of the impulses the experiment is of the same nature. The preliminary sections are the same, and the hemisection is made in the same place; but in this case the cord is split from below. The first cut is made well below the known lower limit of the crossing, and the splitting is continued up toward the hemisection until stimulation of the cord above the hemisection is no longer effective. The upper limit of the crossing of impulses in the cord was thus shown to be a point just below the origin of the strands which make up the II lumbar root, a point identical with that which was determined to be the upper level of the reflex centre. This determination was made separately for the impulses carried by the sympathetic fibres, for those carried by the sacral nerves, and for those aroused reflexly by stimulation of the sciatic nerves.

With the impulses carried by the lumbar nerves, however, a further determination was made. It has been shown by Langley and Anderson[40] (p. 188),[41] (p. 423),[48] (p. 73) that there is a crossing of impulses in the inferior mesenteric ganglion, — a fact easily demonstrated by cutting one hypogastric nerve and stimulating the peripheral cut ends of the sympathetic rami to the ganglion on each side separately. A series of experiments was therefore necessary to ascertain whether impulses crossing in the cord are identical with the ones which pass the inferior mesenteric ganglion uncrossed; or in other words, to determine whether the partial crossing in the inferior mesenteric ganglion bears any relation to the crossing of impulses in the lumbar cord. The accompanying figure (Fig. 3) illustrates diagrammatically the four possible paths open for impulses.

By making a right lateral hemisection of the lower dorsal cord, and stimulating only above the hemisection, impulses of the left side were isolated. The four possible paths, as shown in the figure, were then provided for by making sections between the cord and the ganglion, and between the ganglion and the bladder, as indicated by the blocks in the figure. The sacral nerves were thrown out by a complete transverse section of the cord between the VI and VII lumbar roots. It was demonstrated that each of the four possible paths is taken. The crossing in the inferior mesenteric ganglion, therefore, cannot be regarded as supplementing the incomplete crossing of impulses which takes place in the cord.

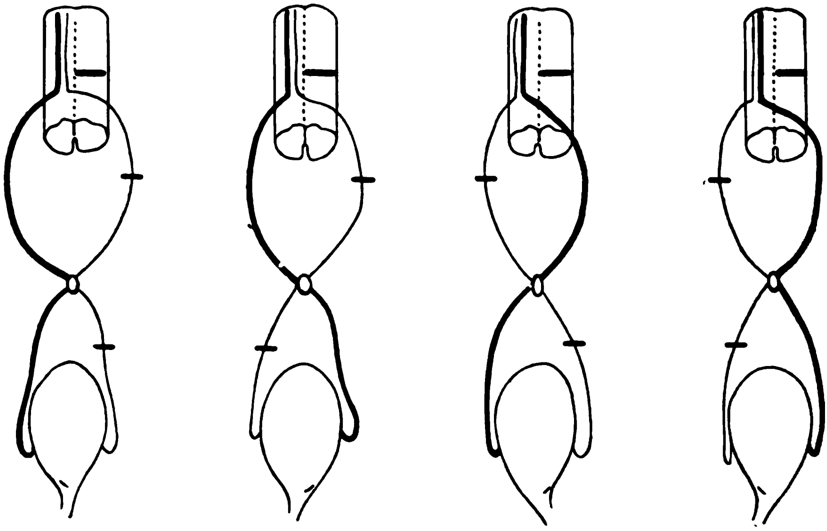

FIGURE 3. Diagram to illustrate the four possible paths open for impulses leaving the cord by the lumbar spinal roots, and passing through the rami to the inferior mesenteric ganglion, and through the hypogastric nerves to the bladder.

The experiments thus far have determined only the limits of the crossing of impulses which occurs below the XII dorsal root. The cord above that level still remains to be explored. It may be shown, by making a right lateral hemisection at the XII dorsal and a left lateral one at the first cervical root, and stimulating above the upper hemisection, that no impulses cross the median line between the two hemisections. In this experiment it is necessary to stimulate the cord by means of punctures, for the danger of spreading makes results obtained with electrical stimuli valueless. The effect of stimulation by punctures above the first cervical root before the hemisections are made is to produce a strong contraction of the bladder. After the first hemisection at the XII dorsal root the contraction is considerably weaker; after the second hemisection at the first cervical root on the opposite side, no contraction at all can be obtained.

Above the first cervical root there is a certain variable amount of crossing. If the cord be hemisected anywhere in the cervical or dorsal regions, stimulation by puncture of the extreme upper part of the cord is effective on either side. If for example a right lateral hemisection be made at the first cervical root, then puncturing on either side above the hemisection produces contraction of the bladder. If the cord be then split from the first cervical root to the calamus scriptorius in the middle line, stimulation on the right side above the hemisection no longer produces any effect. This crossing between the medulla and the first cervical root is probably the lower part of a crossing which takes place in the decussation of the pyramids. It is of the same extent for impulses carried by the sympathetic fibres as for those carried by the sacral nerves.

With regard to the exact nature of the relation between the two halves of the spinal centre very little can be said. Griffiths[45] in referring to the centres of Budge and Gianuzzi, says: "I am inclined to go further, and even suggest that this centre is bilateral, each half controlling, as does each nerve, the corresponding half of the bladder; and that each half is connected by commissural fibres, so that under ordinary circumstances the two halves act in harmony with one another, and are influenced equally by stimuli transmitted to one or the other. The respiratory centre is just of such a nature. This view I have not yet subjected to experimental verification, but I hope soon to do so" (pp. 270 and 271).

The fact that the cord may be split in the middle line with only ordinary care, without interfering with any but crossed impulses, certainly shows that the vesical centre is distinctly bilateral. But that each half controls the corresponding half of the bladder is not true of impulses carried by the lumbar nerves. Each half of the centre controls both halves of the bladder by means of a crossing of impulses in the inferior mesenteric ganglion. Nor is there anything to show that the cells of one half of the centre do not send fibres through the sacral nerves of both sides.

THE PATH OF THE IMPULSES IN THE CORD.

Budge[2] in 1841 (p. 159) noted that stimulation of the anterior half of the cord was especially effective in producing bladder contractions, and in 1864[7] described an experiment in which the posterior half of the cord was removed, stimulation of the anterior half producing contraction of the bladder (p. 15). Mosso and Pellacani[28] (p. 293),[27] (p. 36) demonstrated that the fibres for the bladder run in the posterior columns and in the posterior part of the lateral columns, and Ott[37] (p. 412) found that impulses pass to the bladder in the lateral columns.

Above the vesical centre it has been found that impulses pass down the cord in the posterior half of the lateral columns only. A right lateral hemisection of the cord was made and stimulation above the cut was shown to be effective. A cut was then made on the left side which severed the remainder of the dorsal half of the cord with the exception of the part occupied by the posterior columns. Stimulation above the point of partial section was then entirely without effect, while below the cut stimulation still produced a forcible bladder contraction. Again, the whole anterior half of the cord was cut without stopping the passage of impulses. In another case the middle third was severed, so as to interrupt the posterior and anterior columns, leaving the lateral ones intact, without interfering with the conduction of impulses. In still another experiment a complete section was made in the dorsal cord, and a portion five or six centimetres long, lying in a pool of warm saline solution, was split longitudinally in such a way that the dorsal half of the lateral column on one side was isolated. On tying a ligature around the upper end of this strip and raising it well above the pool in which the remainder of the cord still lay, stimulation produced marked bladder contractions.

Below the level of the centre the impulses were shown to pass in the lateral columns. If the lateral columns of the cord were cut at the V or VI lumbar root, leaving only the anterior and posterior columns intact, stimulation of the cord in the dorsal region failed to produce any effect after the lumbar nerves to the bladder were interrupted. And again, section of the middle third of the cord at the level of the VI lumbar root fails to interrupt the impulses carried by the sacral roots, or those aroused by stimulating the sciatic nerves. Since sections in the lower lumbar cord interfere more or less with the afferent fibres of the sciatic nerve, the fact that section of the lateral columns in that region will block motor impulses aroused reflexly by stimulating the sciatics was demonstrated in another way. The sacral supply to the hypogastric plexus was cut on the right side and the right sciatic nerve was stimulated. A crossed reflex was thus obtained. The lateral column of the left side was then cut at the VI lumbar root, and the reflex contraction was abolished. The afferent elements in the reflex were thus left uninjured while the efferent fibres were shown to lie in the lateral columns.

CONCLUSIONS.

It has been shown, in confirmation of earlier work, that stimulation either electrically, mechanically, or thermally, of the motor nerve supply of the bladder, causes a contraction of the whole musculature of the viscus. Stimuli sufficient for the cord and for the sacral nerves are much weaker than those which must be used to stimulate the sympathetic supply. The irritability of the sacral nerves persists much longer than that of the hypogastrics after the heart ceases to beat, the sympathetic rami to the inferior mesenteric ganglion failing almost at once. The effect of stimulating the nerves of the sacral group is to cause a powerful contraction of the bladder, followed by a slow relaxation. The effect of stimulating the sympathetic nerves, on the other hand, is to produce a smaller contraction, followed in nearly every case by a dilatation of much greater extent. Impulses reaching the bladder through one hypogastric nerve, or through the sacral supply of one side, cause contraction of that half of the bladder only. There are sensory bladder nerves in the sacral supply, with reflex centres in the cord and in the hypogastric plexus. The sensory fibres for the bladder which pass up the hypogastric nerves have their reflex centre in the inferior mesenteric ganglion. Finally, the rhythmical contractions of the bladder are much smaller than those produced experimentally.

The further results described in the foregoing paper may be summed up as follows: —

The vesico-motor impulses passing down one side of the cord do not cross the median line of the cord below the first cervical root except in the region of the spinal vesical centre.

Impulses cross between the lower point of origin of the II lumbar root and the lower point of origin of the V lumbar; and there is a certain amount of crossing between the lower limit of the medulla and the first cervical root.

The crossing of impulses, identical in extent for those which leave by sacral or by sympathetic fibres, occurs between these same limits for impulses aroused reflexly by stimulating the sciatic nerves.

The upper limit of the reflex centre is the same as the upper limit of the crossing of the impulses.

The path in the cord of all vesico-motor impulses above the centre is in the posterior part of the lateral columns only. Below the centre the impulses pass in the lateral columns.

Impulses coming down one side of the cord pass out by the lumbar nerves of both sides, and by the sacral nerves of both sides.

Impulses leaving the cord by the lumbar roots of one side pass to the bladder by the hypogastric nerves of both sides. There is a second crossing of impulses in the inferior mesenteric ganglion, and all four possible paths are taken.

The motor impulses aroused reflexly by stimulating the sciatic nerve on one side pass out by the sacral nerves of both sides.

The vesical centre is bilateral, each half controlling both halves of the bladder at least through the lumbar nerves.

The motor fibres which carry the impulses aroused reflexly by stimulating the sacral sensory fibres lie wholly within the sacral nerves.

The motor fibres which convey the impulses aroused by stimulating the central cut end of the sciatic nerve, lie wholly in the sacral supply to the bladder.

The sympathetic rami to the ganglion have not been shown to contain any sensory fibres for the bladder.

The usual effect of stimulating the cord with all the peripheral nerves intact is the same as when the sacral nerves alone are stimulated; on excluding the sacral nerves the effect is the same as when the lumbar supply alone is stimulated.

A frequent effect of stimulation through the cord, and also through the peripheral nerves, is to produce dilatation of a previously contracted bladder.

Sometimes a single stimulation, lasting only long enough to cause a single contraction, gives rise to a rhythmical series of contractions or oscillations of gradually diminishing extent.

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ON SECRETORY NERVES TO THE SUPRARENAL CAPSULES.

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THE more exact study of the physiological effects of intravenous injections of extracts of the suprarenal glands marks the beginning of a new epoch in the history of these structures. These effects, studied and described independently and simultaneously by Cybulski and Szymonowicz[1] on the one hand and by Oliver and Schäfer[2] on the other, are now well known. In a normal animal, they consist in a pronounced slowing of the heart and a rise of blood pressure; in animals whose vagi have been thrown out of function either by section or by atropine, in heart acceleration and still greater rise of blood pressure.

The significance of the marked reactions exhibited by suprarenal extracts depends very largely upon the possibility of proving that the substance producing them is formed normally within the gland. It is conceivable, — indeed, it has been claimed,[3] — that the substance is formed in the dead gland as the result of post-mortem changes, and under the normal conditions of life does not exist. Cybulski first succeeded in demonstrating its origin in the living gland when he discovered that the blood collected from the adrenal vein gives a reaction similar to that of the substance itself, while blood from other veins does not possess this property.

Cybulski apparently made but few experiments of this kind. Langlois[4] succeeded in a single trial in repeating Cybulski's observation, while Oliver and Schäfer[5] state that in three attempts they were unable to arrive at this result. In the spring of 1897 I undertook a series of experiments for the purpose of accumulating evidence on a point of such fundamental importance, and was soon convinced that Cybulski's observation was correct.[1] Further details of these experiments have been withheld for the reason that additional ones were in progress on a new point in the physiology of these same organs. Given the means of collecting the product of the normal activity of the gland in the blood issuing from its vein and of testing quantitatively the amount of the secretion in terms of characteristic physiological effects, the opportunity is presented of determining whether this secretory function is under nervous control, as the activity of the glands with external secretions is known to be. This question has but recently been raised with reference to the internal secretions, and its pursuit has yielded no result thus far for various reasons.

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[1] CYBULSKI and SZYMONOWICZ: Gazeta Lekarska, 1895, p. 299; abstract in Jahresberichte der Thier-Chemie, 1895, p. 379; SZYMONOWICZ: Archiv f. d. ges. Physiol., 1896, lxiv, p. 97; CYBULSKI: Sitzungsber. d. Akad. d. Wissensch. in Krakau, 1895, March 4.

[2] OLIVER and SCHÄFER: Journal of physiology, 1895; also SCHÄFER: Textbook of physiology, 1898, i, p. 958.

[3] TIZZONI: quoted by LANGLOIS: Revue scientifique, 1897, p. 303.

[4] LANGLOIS: Revue scientifique, 1897, p. 303.

[5] OLIVER and SCHÄFER: *loc. cit;* p. 275.

In the case of the suprarenal glands the conditions for investigating this point are peculiarly favorable, partly because of convenient anatomical relations, partly owing to the fact that here one has to deal with a secretion possessing specific reactions that can be readily recognized. It was this question of the existence of secretory nerves to the suprarenals to which my attention was from the first mainly directed, and the confirmation of Cybulski's discovery of the presence of the adrenal substance in the blood of its vein was simply a preliminary measure. The appearance of A. Biedl's[2] work has rendered the publication of the details of my first series of experiments unnecessary. In the second part of Biedl's paper he too confirmed the discovery of Cybulski, and then went on independently to investigate the influence of the nervous system on the internal secretion of the adrenals. As the result of stimulation of the splanchnic nerve in the thorax, he describes certain slight and rather vague morphological changes in the adrenal blood which seem to him to indicate the existence of secretory fibres. The experiments in which he compared the effects of injections of adrenal bloods collected before and during stimulation of the nerve gave only negative results, and he was compelled to acknowledge that his work had furnished no satisfactory evidence in support of this conclusion. My experiments on this point had been practically concluded when Biedl's work came to my notice in the fall of 1897. As the method used by us was so similar in all essential respects and gave me results very different from his, I have since multiplied the number of my experiments in order to make the evidence as conclusive as possible. It is the result of these experiments bearing on the existence of secretory nerves to the suprarenal capsules that I desire to set forth briefly in the following pages.

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[1] This result was reported to the physiological section at the meeting of the Congress of American Physicians and Surgeons held at Washington in 1897, brief notices of the report appearing subsequently in Science, 1897, p. 904, and in the Johns Hopkins University Circular, 1897, p. 49.

[2] BIEDL, A: Archiv f. d. ges. Physiol., 1897, lxvii, p. 443.

METHOD.

The experiments involve two separate and distinct steps. The first consists in the collection of the blood to be tested; the second in the study of the effects of the intravenous injection of this blood into the same or into another animal. On account of their convenient size, dogs were used throughout. In every case special care was taken to have them completely anæsthetized with morphia and ether. To collect the adrenal blood, the abdomen was opened widely by means of generous longitudinal and transverse incisions, and the viscera immediately protected with sponges saturated with warm normal saline solution. Pushing the viscera over to the right, the left adrenal gland was exposed and the vein quickly prepared. A ligature was placed around the vein on either side of the gland, the one on the mesial side serving to tie off the central end of the vein, the other, on the lateral side, being used to tie in the cannula. This cannula was simply a straight glass tube about twelve centimetres long. When in position it lay horizontally and reached several centimetres beyond the body wall. The blood dropped from the free end of the cannula directly into the collecting vessels. The ligature on the central end of the vein prevented any reflux from the vena cava, and that around the neck of the cannula, being close to the gland, hindered the admixture of any blood from the numerous muscular branches joining the capsular vein. The blood issuing from the cannula was therefore derived from the gland alone. When the blood clotted in the cannula, and this occurred the more frequently the tardier the outflow, the clot was easily removed by introducing a fine straw. The splanchnic nerve was prepared from the point where it emerges from under the diaphragm to where it sends off its branches to the adrenal gland and renal plexus, and after placing a tight ligature around it, as high as possible, was arranged upon shielded electrodes. A kymographic record taken from the carotid artery served the twofold purpose of indicating the condition of the animal and of controlling the stimulation of the nerve. The interrupted current from an ordinary induction coil was used to stimulate the nerve, and in a given stimulation period the current was alternately turned into and taken out of the nerve for approximately equal intervals of ten to fifteen seconds. The current was made strong enough to give a decided rise of blood pressure with each application; so long as this rise occurred it was taken for granted that the irritability of the nerve persisted and that any secretory fibres contained in it would respond.

The following specimens of blood were collected in every complete experiment: *a*, from the femoral vein; *b*, from the adrenal vein before stimulation; *c*, from the adrenal vein during stimulation. In a few cases the condition of the animal continued good enough to enable a number of additional specimens to be obtained. These included: *d*, from the adrenal vein after stimulation; *e*, from the adrenal vein during a second stimulation period; *f*, from the adrenal vein in the second interval, that is to say, in the period succeeding the second stimulation. The time consumed in collecting each specimen naturally varied with the rate of outflow. The object was to obtain sufficient quantities of each kind of blood for the subsequent injections; and as experience soon taught that 30 c.c. or more of blood were required for each dose to ensure positive results in dogs, enough time was allowed to collect 75 to 100 c.c. of each kind. The actual times required for this are given in the tabulated protocols of the experiments. They varied between fifteen and seventy minutes. It goes without saying that quantities of blood so large as these can be best obtained from large animals, and such were accordingly selected for this purpose. Small dogs, on the contrary, are better adapted for testing the reaction, since for a given volume of blood injected, the smaller the dog the larger relatively will be the dose of adrenal substance. The several lots of blood were defibrinated and filtered through muslin. It should be stated that the femoral blood taken previous to opening the abdomen was used in every experiment as a control upon the normal adrenal blood.

For the second part of the experiment a fresh dog likewise under morphia and ether, was generally used. By the time the necessary blood was obtained from the first dog, its general condition was unfavorable for good reactions. The animal was connected with the kymograph in the usual manner for a blood pressure record, and the bloods to be tested were injected into the external jugular vein at suitable intervals. In all the later experiments provision was made to have the blood to be injected raised to as near the body-temperature as possible. Whenever the supply of blood permitted, the injections were made in two series, the first while the vagi remained intact, the second after double vagotomy or after the administration of five milligrams of atropine. At the end of the second series, normal salt solution in amounts equal to the doses of blood previously used was injected in order to eliminate any possible effect of the mere introduction of that amount of fluid into the circulation; and finally by means of adrenal extract it was determined whether the physiological mechanisms on which the reaction depends were still functional at that time.

RESULTS.

As stated above, these experiments were intended to determine whether the activity of the suprarenal glands is under the influence of secretory nerves. The specific constituent of their secretion which for convenience may be spoken of provisionally as the " adrenal substance " until the chemists succeed in isolating and identifying it, we now know passes directly from the gland into the blood stream, and its presence there can usually be demonstrated with ease by collecting the blood as it flows from the gland vein and injecting it into the same or another animal. In dogs 10 c.c. of adrenal blood or even less may at times suffice for this demonstration; but the result is more certain with 30 c.c. or more. Whenever the amount is large enough, and this naturally depends on the concentration in the active principle, the result will be a change in heart-rate and blood pressure similar to that produced by extracts of the gland. If the suprarenal glands are supplied with secretory fibres whose stimulation augments the production of adrenal substance, evidence of the same should be furnished in the increased concentration of the blood attendant upon their stimulation, and in the correspondingly heightened effects of this blood upon the heart and blood vessels. The nervous connections of the suprarenal glands in the dog have been figured and described by Jacobi.[1] According to him the suprarenals might obtain secretory fibres from several sources, seeing that they are intimately connected with the solar and renal plexuses as well as with the splanchnic nerve. In my experiments the latter nerve alone was stimulated to obtain secretory effects upon the gland. Figures 1 and 2 will serve as illustrations of the result obtained. Figure 1 presents two blood pressure

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[1] JACOBI: Arch. f. exper. Pathol. u. Pharmakol., 1891, p. 171.

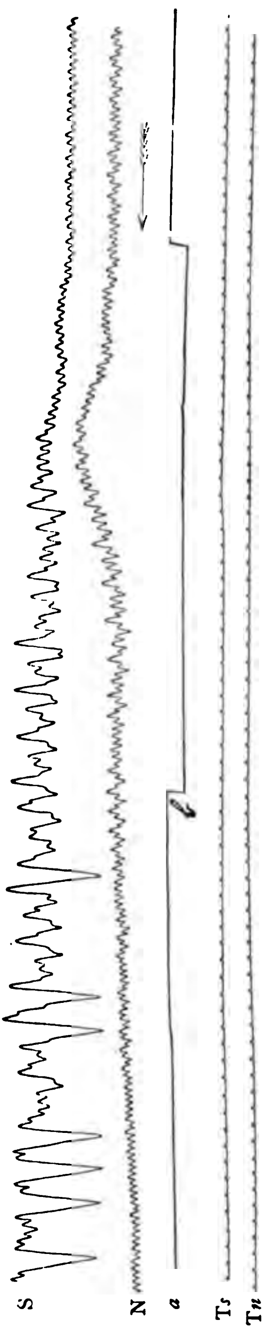

FIGURE 1. One third original size. To be read from right to left. The effect on the blood pressure in a dog with intact vagi of injecting 35 c.c. of blood taken from the adrenal vein of another dog before (curve N) and during (S) stimulation of the splanchnic nerve. An interval of ten minutes separates the two curves. The break (*b*) in the line *a* measures the duration of injection (32 sec.). T*n* and T*s* are the base-lines of curves N and S, respectively; they also give the time in seconds.

tracings from a dog with intact vagus nerves taken at an interval of about ten minutes. N shows the effect of 35 c.c. normal adrenal blood, S that of the same amount of stimulation blood. The blood in both cases was injected slowly into the external jugular vein at body-temperature, the injection occupying 32 seconds, as indicated by the break (*b*) in the line *a*. Each blood pressure curve is accompanied by its own base-line, which also gives the time in seconds. T*n* belongs to the curve N, T*s* to S. These records are part of Experiment V, May 20, 1898. Figure 2 gives similar records from Experiment VIII, May 31, 1898. In this case the amount of each kind of blood was 65 c.c. and the injections were made after the dog had received a full dose of atropine. The vagi were accordingly completely paralyzed, and the effects consist in rise of blood pressure and acceleration. As in Fig. 1, the curve S is the record for the stimulation blood, N that for normal blood, *a* gives the time and duration of the injection, while T*s* and T*n* are the respective base-lines. These figures show plainly that a given bulk of adrenal blood taken during stimulation has a decidedly greater effect than the same bulk of normal adrenal blood, meaning by "normal" blood that which was taken when not stimulating. With the vagi intact in the reaction dog, this augmented effect is particularly manifest in the more pronounced inhibition of the heart (see Fig. 1). In atropinized dogs, the stimulation blood gives a greater rise of blood pressure and more extensive acceleration.

Biedl[1] has shown, however, that the splanchnic nerve contains vasodilator fibres for the adrenal, so that stimulation of its trunk must materially modify its blood-supply. Before the increased efficacy of the blood noted above as resulting from stimulation of this nerve can be interpreted as indicating the existence of secretory nerves, these accompanying vasomotor disturbances and their possible influence on the gland must be taken into account. To determine the value of this factor so far as my result is concerned, I have arranged my results in tabular form (page 210) so as to show side by side with the effect of any given specimen of blood the condition of the circulation prevailing at the time of its collection. It will serve practically as a brief protocol of the several experiments.

The injections were made in dogs in which the vagi were intact or in which the inhibitory influence of these nerves had

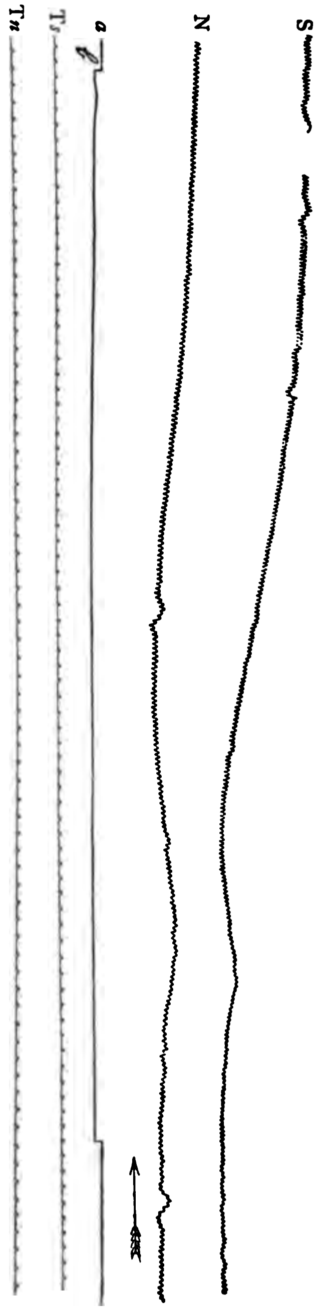

FIGURE 2. One third original size. To be read from right to left. The effect on the blood pressure in a dog with paralyzed vagi of injecting 65 c.c. of blood taken from the adrenal vein of another dog before (curve N) and during (S) stimulation of the splanchnic nerve. The break (*b*) in the line *a* measures the duration of injection. T*n* and T*s* are the base-lines of curves N and S, respectively; they also give the time in seconds.

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[1] BIEDL: Archiv f. d. ges. Physiol., 1897, lxvii, p. 443.

1 No. of Exp.	2 Condition of Vagi.	3 Amount and character of injections.								4 Diminution (−) or increase (+) in the Heart-rate per minute.							
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*
I	Intact	25	25	25	....	....	....	....	....	−57	−48	−36	....	....	....	....	....
	Atropine	30	35	25	....	....	....	....	10	−16	−20	−16	....	....	....	....	−15
II	Atropine	35	40	40	....	....	....	....	10	− 9	+ 8	+63	....	....	....	....	+48
III	Cut	16	38	45	....	....	....	....	5	−15	0	+ 6	....	....	....	....	+33
		32°	35°	32°.5	....	....	....	....	....	....	....	....	....	....	....	....	....
IV	Intact	35	35	35	....	....	....	....	....	−29	−14	−32	....	....	....	....	....
		38°	39°	39°	....	....	....	....	....	....	....	....	....	....	....	....	....
V	Intact	35	35	35	....	....	....	....	....	+15	−18	−36	....	....	....	....	....
Fig. 1		39°	38°.5	38.°5	....	....	....	....	....	....	....	....	....	....	....	....	....
	Atropine	35	35	35	30	....	....	35	20	+ 8	+66	+66	+45	....	....	0	+72
		38°	39°	39°	40°	....	....	39°	39°	....	....	....	....	....	....	....	....
VI	Intact	50	50	50	50	50	50	....	....	−12	−12	−24	−14	−20	−16	....	....
		39°	39°	38°	38°.5	38°	39°	....	....	....	....	....	....	....	....	....	....
	Atropine	50	50	50	50	50	50	50	50	0	0	+18	0	+13	+ 9	0	+51
		39°.5	39°.5	39°	38°	38°.5	39°	39°	39°.5	....	....	....	....	....	....	....	....
VII	Atropine	35	40	40	35	35	....	40	25	0	0	0	0	+28	....	0	+51
		39°	39°	39°	38°.5	39°	....	39°.5	....	....	....	....	....	....	....	....	....
VIII	Atropine	65	65	65	....	....	....	65	20	−10	+23	+36	....	....	....	0	+80
Fig. 2		39°.5	39	39	....	....	....	39°.5	....	....	....	....	....	....	....	....	....

5								6
Diminution (−) or increase (+) in Blood Pressure, in millimetres of mercury.								General Data.
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	
+ 7 +10	+10 +22	+21 +36	....	....	....	....	.... + 95	Stimulation of Splanchnic caused rise of blood pressure and acceleration of outflow from adrenal vein. Blood collected included — *a* 60 c.c. *b* 65 c.c. in 25 min. Aver. rate = 156 c.c. per hr. *c* 80 c.c. " 70 " " " 68 c.c. "
−10	+ 4	+53	....	....	....	....	+ 63	Stimulation of Splanchnic gave same effect as in Exper. I. Blood collected included — *a* 65 c.c. *b* 75 c.c. in 30 min. Aver. rate = 150 c.c. per hr. *c* 105 c.c. " 55 " " " 120 c.c. "
+ 8	+20	+41	....	....	....	....	+ 56	Stimulation of Splanchnic same as above. Blood collected included — *a* 90 c.c. *b* 70 c.c. in 30 min. Aver. rate = 140 c.c. per hr. *c* 85 c.c. " 30 " " " 170 c.c. "
0	+ 7	+26	....	....	....	....	....	Stimulation of Splanchnic as above. Blood collected included — *a* 50 c.c. *b* 50 c.c. in 30 min. Aver. rate = 100 c.c. per hr. *c* 75 c.c. " 30 " " " 150 c.c. "
+19 .... 0	+11 .... +58	+50 .... +61	.... +54	....	....	.... + 6	.... + 80	Stimulation of Splanchnic as before. Blood collected included — *a* 85 c.c. *b* 100 c.c. in 40 min. Aver. rate = 150 c.c. per hr. *c* 75 c.c. " 45 " " " 100 c.c. " *d* 30 c.c. " 50 " " " 36 c.c. "
+13 .... +10	+ 6 .... +12	+13 .... +41	+ 7 .... +13	+14 .... +35	+ 7 .... +30	.... + 4	.... +104	Stimulation of Splanchnic as before. Blood collected included — *a* 135 c.c. *b* 165 c.c. in 23 min. Aver. rate = 432 c.c. per hr. *c* 125 c.c. " 18 " " " 420 c.c. " *d* 100 c.c. " 15 " " " 400 c.c. " *e* 135 c.c. " 20 " " " 405 c.c. " *f* 125 c.c. " 60 " " " 125 c.c. "
+ 4	+10	+12	+ 4	+34	....	0	+ 66	Stimulation of Splanchnic as before. Blood collected included — *a* 100 c.c. *b* 145 c.c. in 35 min. Aver. rate = 248 c.c. per hr. *c* 110 c.c. " 25 " " " 264 c.c. " *d* 60 c.c. " 30 " " " 120 c.c. " *e* 35 c.c. " 20 " " " 105 c.c. "
+24	+22	+68	....	....	....	+ 6	+161	Stimulation of Splanchnic as before. Blood collected included — *a* 75 c.c. *b* 90 c.c. in 35 min. Aver. rate = 155 c.c. per hr. *c* 100 c.c. " 30 " " " 200 c.c. "

been abolished either by section or by poisoning with atropine (Column 2, page 210). Wherever atropine was employed it is to be understood that the drug was given in sufficient quantity to paralyze completely the inhibitory fibres before the injections to be tested were made.

In Exp. I the reaction dog furnished the blood used for injection; in the remaining experiments the blood in each instance was drawn from another animal.

It will be observed that Columns 3, 4, and 5 are each subdivided into eight subsidiary ones, headed respectively by the first eight letters of the alphabet. These letters have the same significance wherever they occur in this paper, and refer in each case to a blood-specimen or other reagent tested on the reaction dog. *a*, means blood from the femoral vein; *b*, blood from the adrenal vein previous to stimulation of the splanchnic nerve; *c*, blood from the adrenal vein during stimulation or, briefly, 1st stimulation blood; *d*, blood from the adrenal vein after the first stimulation period or, briefly, 1st interval blood; *e*, blood from the adrenal vein during a second stimulation period, or 2d stimulation blood; *f*, blood from the adrenal vein during the 2d interval; *g*, normal saline solution; *h*, extract of adrenal gland of no definite concentration, the figures giving simply the volume of the injection. In Column 3 the temperature of the injection is indicated by figures standing immediately beneath those giving the volume; where no temperature is given, it is to be understood that the injection was made at room temperature. The figures in Column 4 give the changes in the heart-rate per minute resulting from the injection. A minus sign in front of a number indicates inhibition; a plus sign, acceleration. The blood pressure changes (Column 5) are recorded in millimetres of mercury, the minus sign here signifying a fall of blood pressure; a plus sign, rise of pressure for so many millimetres of mercury. In respect to both heart-rate and blood pressure the figures give the maximal effect for the injection, and were obtained by taking the algebraic difference between the pulse-rate and blood pressure for the ten second period just preceding an injection, and the pulse-rate and blood pressure, respectively, for that ten second interval after the injection when the effect was greatest. Lastly, Column 6 furnishes certain miscellaneous general data of more or less importance to the correct interpretation of the result. The remarks being written out in full will explain themselves.

Effects of "b" and "c" compared, when injected into dogs with intact vagi. — Owing to the lack of sufficient quantities of blood, it frequently happened that only a single series of injections could be made. In such cases, they were preferably made into dogs whose vagi had been thrown out of function, as it seemed to me that there was a little more certainty of detecting and estimating small differences in the blood pressure changes under these circumstances than when complicated by inhibitory effects on the heart. For this reason the table contains only four comparative tests on dogs with intact vagus nerves. These occur in Experiments I, IV, V, and VI. While the doses of normal and stimulation blood in each case were equal, it will be seen that the stimulation blood throughout gave the greater rise of pressure and except in Experiment I a greater inhibition. The difference in favor of the stimulation blood is moreover well-marked, ranging from one hundred per cent upwards. Thus, in

Exp. I.	25 c.c. *b*	slowed heart	48	per min.	and raised	pressure	10	mm. Hg.
	25 c.c. *c*	"	36	"	"	"	21	"
" IV.	35 c.c. *b*	"	14	"	"	"	7	"
	35 c.c. *c*	"	32	"	"	"	26	"
" V.	35 c.c. *b*	"	18	"	"	"	11	"
	35 c.c. *c*	"	36	"	"	"	50	"
" VI.	50 c.c. *b*	"	12	"	"	"	6	"
	50 c.c. *c*	"	24	"	"	"	13	"

The exceptional result so far as the heart-rate is concerned in Exp. I is, in all probability, accounted for by the fact that in this case the blood was injected at room temperature. This being considerably below that of the dog (approximately 20° C.), it is evident that the direct effect of the low temperature on the heart must enter as a disturbing factor, the extent of this influence varying with the rapidity of the injection. That this is the correct explanation is further indicated by the fact that the vaso-constriction and the resultant rise of blood pressure, upon which the temperature difference could have no effect, was even in this case greater for the stimulation blood than for the normal. The unusually marked slowing of the heart observed in this experiment must therefore be regarded as the summated effect of cold and of adrenal substance coöperating to produce a change in the same direction. Compare also the results given below obtained on the same dog after the exhibition of atropine.

Effects of "b" and "c" compared, when injected into dogs after section or complete paralysis of both vagi.—The number of observations of this class was considerably larger, but the results were equally uniform and in the same sense. Thus, in

Exp.	I.	35 c.c. *b*	slowed heart	20	per min. and raised pressure	22	mm. Hg.
		25 c.c. *c*	“	16	“ “ “	36	“
“	II.	40 c.c. *b*	quickened heart	8	per min. and raised press.	4	“
		40 c.c. *c*	“	63	“ “ “	53	“
“	III.	38 c.c. *b*	“	0	“ “ “	20	“
		45 c.c. *c*	“	6	“ “ “	41	“
“	V.	35 c.c. *b*	“	66	“ “ “	58	“
		35 c.c. *c*	“	66	“ “ “	61	“
		30 c.c. *d*	“	45	“ “ “	54	“
“	VI.	50 c.c. *b*	“	0	“ “ “	12	“
		50 c.c. *c*	“	18	“ “ “	41	“
		50 c.c. *d*	“	0	“ “ “	13	“
		50 c.c. *e*	“	13	“ “ “	35	“
		50 c.c. *f*	“	9	“ “ “	30	“
“	VII.	40 c.c. *b*	“	0	“ “ “	10	“
		40 c.c. *c*	“	0	“ “ “	12	“
		35 c.c. *d*	“	0	“ “ “	4	“
		35 c.c. *e*	“	28	“ “ “	34	“
“	VIII.	65 c.c. *b*	“	23	“ “ “	22	“
		65 c.c. *c*	“	36	“ “ “	68	“

In this series Experiment I again presents the only exception in that it gives a slowing of the heart for both normal and stimulation blood where acceleration should occur. This result, however, only confirms what was said above concerning the part played by the low temperature at which the blood in this experiment was injected. The retarding influence of the cold acting on the heart directly simply over-compensated the accelerating tendency of the adrenal blood. In the remaining experiments the stimulation blood either equalled or surpassed the normal blood both as to the effect on the heart and on the peripheral vessels. Experiment VI furnishes a particularly interesting and striking illustration of the influence which stimulation of the splanchnic nerve has on the content of adrenal substance in the blood. In this experiment I was able to continue the collection of blood through five alternate periods of rest and stimulation, obtaining altogether 650 c.c. of adrenal blood. The table shows a distinct increase in the concentration of the blood during each stimulation period, followed by a diminution in the intervening periods of rest. In Experiment VII a similar series was obtained; but the concentration being low throughout, the result is less conspicuous. In this connection it may be interesting to call attention to the extended variation in the concentration as regards adrenal substance observed in normal adrenal blood from different individuals of the same species. From those in which so much as 40 and 50 c.c. of blood gave no acceleration and only 10 and 12 mm. rise of pressure respectively, they range in my table to such as gave an acceleration of 66 beats per minute and 58 mm. rise of pressure with 35 c.c. of blood. In fact, I often met with instances in which 10 c.c. still gave a distinct reaction, as recorded also by others engaged upon this problem. In these individuals with exceptionally high concentration of the normal adrenal blood, stimulation of the nerve failed to produce the usual increase, suggesting the idea that in them the gland was already at the maximum height of secretion. Nothing can be stated at present concerning the normal mechanism through which this secretion is regulated; but every analogy would lead us to expect these very fluctuations in an apparatus of such a seemingly important function. It seems to me also that these normal variations may account for the negative result obtained by Oliver and Schäfer. They probably met with slowly secreting glands, and used an insufficient amount of the relatively dilute blood. In my experiments, in which comparatively large volumes of blood were used, the adrenal effect was never completely absent, however small it may have been in several instances. For example, in Experiments VI and VII, in which the volumes used were respectively 50 and 40 c.c., there was still a rise of 12 and 10 mm. in the blood pressure, while the heart-rate continued unchanged. In spite of the slight extent of this change, it must be regarded as a genuine adrenal effect because the same volumes of femoral vein blood had given still smaller changes just before, and normal salt solution given directly after gave in one case (VI) only 4 mm. rise and in the other (VII) no rise whatever. The result was therefore not due to the mere mechanical effect of that much fluid added to the circulation.

Relation between blood-supply and rate of secretion in the suprarenals.— I have shown in the preceding sections that stimulation of the splanchnic nerve augments adrenal secretion. A point of great interest arises here as to the interpretation to be placed upon this fact. Is the augmentation to be ascribed to a direct influence of the nerve on the metabolism of the secreting cells, or is it indirectly produced by the accompanying vascular phenomena? Increased functional activity is commonly associated with vascular dilatation and increased blood-flow, and in some organs, for example the kidneys, the vascular change is the direct cause of the functional variation. Does a similar relation obtain for the suprarenals? This is a legitimate question, and if the results showed a regular variation in the outflow from the adrenal vein accompanying the stimulation it would be difficult to reach a conclusion.

It is well known that stimulation of the splanchnic nerve does produce extensive vasomotor changes. In my experiments the customary rise of general arterial pressure produced by such stimulation was regularly obtained. In fact, as previously stated, this reaction was relied upon to indicate the maintenance of irritability in the nerve. This general rise of pressure in itself would tend to increase the blood-supply to the adrenal, provided its intrinsic arteries did not participitate in the constriction. Far from being constricted Biedl[1] has shown that they actively dilate on account of the vasodilator fibres supplied to the gland in the splanchnic trunk. Local dilatation simultaneous with general constriction constitute the most favorable condition for increasing the blood-supply to an organ, and one should expect the outflow from the adrenal vein to show a marked acceleration in the stimulation periods. As a matter of fact Table I presents examples of every possible relation between the rate of outflow and the increased functional activity attendant upon stimulation of the nerve. The average outflow in a given stimulation period was sometimes the same as in the preceding period of rest, and when it was changed, the change was as frequently retardation as acceleration. The reason for this is not far to seek. It must be remembered that the conditions of the experiment necessarily induce a steady decline of blood pressure in the dog from which the blood is being collected. This follows not only from the extensive abstraction of blood (ranging from 150 c.c. to over 700 c.c.) but is due also in part to the unavoidable partial exposure of the abdominal viscera with its accompanying fall of temperature and increasing vascular paralysis. In two successive half-hour periods, therefore, regularly less blood should be obtained in the second than in the first, independently of any incidental stimulation. Together with this fact must be considered the manner of stimulation employed in these experiments. A so-called stimulation period extending over 18 to 70 minutes embraces alternating 10 to 15 second intervals of actual stimulation and rest. With each application of the current, the outflow is decidedly accelerated, frequently as much as one hundred per cent. But it drops back just as quickly in the succeeding interval of rest, and the normal rate to which it returns constantly diminishes more and more. The average outflow for the whole period, which must be taken as the measure of the blood-supply during that time, is the resultant of two factors whose influence tends in opposite directions. The actual result in a given experiment depends on their relative potency, and so it happens that in one case an increase, in a second a decrease, in a third no change is observed. In passing I may refer also to a third factor which occasionally comes into play, and that is the mechanical interference to the outflow from the development of blood-clots in the vein and cannula. This is especially apt to occur when the outflow is slow, and can be largely avoided by the use of large dogs, as recommended above in another connection.

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[1] BIEDL: Archiv f. d. ges. Physiol., 1897, lxviii, p. 443.

CONCLUSION.

The recent advances in our knowledge of the various glandular organs, especially in that of the several ductless glands, by which a new chapter on "Internal Secretion" has been added to physiology, naturally led to the inquiry as to the dependence of this new function upon specific nerves. The idea of secretory nerves is so familiar in the physiology of glands with an external secretion, that it could not fail to suggest itself in connection with those producing internal secretions. Biedl gives the references to the literature bearing on this point, and shows that as early as 1889 Wyss[1] raised this question with reference to the thyroids. The conception is therefore by no means a new one, although all the credit which he demands should be conceded to Biedl for bringing it prominently to the front. Hitherto no proof for the existence of secretory nerves to glands with an internal secretion has been obtained. So far as the adrenals are concerned, Biedl alone has investigated the point, and with what result may best be stated in his own words. He says, on page 480: "My observations have not yet enabled me to reach the final solution of this problem. It appeared that the blood drawn from the vein during stimulation has no less, or at least no significantly less effect than that drawn previous to stimulation. An increased action was not present." And again, on page 481: "I am fully aware that I was not in a position to bring forward exact evidence for these statements." [1]

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[1] Wyss, A.: Correspondenzblatt für Schweizer Aerzte, 1889, no. 6.

My results recorded in the preceding pages furnish the first demonstration of the actual existence of nerves for controlling an internal secretion. Starting with the fact first discovered by Cybulski and now abundantly confirmed by Langlois, Biedl, and myself, that the internal secretory products of the suprarenals pass directly into the blood stream, it has been shown in the first place that the amount of the active substance can be increased by stimulating the splanchnic nerve below the diaphragm. This conclusion necessarily follows from the fact that the blood from the adrenal vein collected during stimulation of this nerve produces the characteristic adrenal reaction with greater intensity than the blood taken before stimulation, comparing them volume for volume. It has been stated by Biedl[1] on *a priori* grounds that the stimulation blood might be poorer in adrenal substance in spite of heightened activity of the gland because of its more rapid passage through the organ. The same author is also responsible for the statement that with repeated injections of adrenal blood of the same concentration the reaction becomes weaker and weaker. I have made no attempt to verify this last statement, as I regularly injected the stimulation blood after the normal. The stronger reaction with the former must therefore *a fortiori* signify increased concentration if Biedl is right.

Secondly, it has been shown that this effect of splanchnic stimulation can in no way be referred to the indirect effect on the blood-flow through the gland. The increased secretion is observed whether the outflow from the vein increases, decreases, or remains constant. Under these circumstances it is not justifiable to suppose that a causal relation can obtain between the phenomena heightened activity of the gland and rate of blood-flow, and the conclusion that the splanchnic supplies true secretory fibres to the adrenal is extremely probable. The proof is as strong as the method employed is capable of yielding, especially when the number of the experiments and the uniformity of the results are considered. We must look to other methods for corroboration, and perhaps a repetition and extension of

[1] BIEDL: *loc. cit.*, p. 443.

the tests with suitable pharmacological agents will be attended with better success than has been obtained with them hitherto.

SUMMARY OF RESULTS.

1. The active principle of adrenal extract is present in the blood collected from the adrenal vein and constitutes therefore a true internal secretion.

2. The amount of adrenal substance in the adrenal blood as measured by the extent of the physiological effects it is capable of producing is increased by electrical stimulation of the splanchnic nerve below the diaphragm.

3. The increased secretion resulting from splanchnic stimulation is independent of the blood-vascular changes simultaneously provoked.

ON SOME ANALOGIES BETWEEN THE PHYSIOLOGICAL EFFECTS OF HIGH TEMPERATURE, LACK OF OXYGEN, AND CERTAIN POISONS.

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DR. LOEB has expressed the opinion in his lectures that high temperatures and other strong stimulations affect the organism in the way that lack of oxygen does, and it was with the intention of testing the correctness of this supposition that the following series of experiments was made.

The theory that strong stimulation acts like the want of oxygen is based upon Hoppe-Seyler's theory of oxidation.[1] In the cell processes of fermentation take place, by means of which highly reducing substances such as nascent hydrogen are formed. These readily unite with atmospheric oxygen, if it be present, forming oxidized products ($H_2 + O_2 = H_2O + O$) and setting free atoms of oxygen. The free atoms of oxygen are then capable of oxidizing other substances (sugar, lactic acid, etc.) at the low temperature of the animal body. But if oxygen is not present, the nascent hydrogen can reduce the substances in the cell and thereby form new products normally not found in the body. The bodies thus formed may be more or less injurious; in fact, they may act as violent poisons.

It is by means of this theory that Dr. Loeb accounts for the difference which he found in the behavior of the heart of Fundulus and Ctenolabrus when deprived of oxygen.[2] The heart of a Fundulus embryo beats normally about 120 times a minute at a certain temperature. If deprived of oxygen, the rate is reduced within less than an hour to twenty beats, but the heart continues beating at this rate for over twelve hours. The embryo heart of Ctenolabrus, on the other hand, if deprived of oxygen, after a few minutes stops almost suddenly without any considerable previous reduction in the rate. Thus the two hearts differ to a remarkable extent. The stopping of the Ctenolabrus heart can hardly be due to lack of energy, for the

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[1] HOPPE-SEYLER: Physiologische Chemie, 1878, i, pp. 126 *et seq.*

[2] LOEB, J.: Arch. f. d. ges. Physiol., 1896, lxii, pp. 249–294.

muscles of the body still contract after the heart has ceased to beat. In the case of the Fundulus heart, energy for the slow contractions, which continue many hours without oxygen, probably is furnished by fermentation — the more rapid beat, soon reduced by lack of oxygen, is no doubt the result of oxidation; but the stopping of the Ctenolabrus heart is probably not to be explained by the want of material for fermentation. The most reasonable explanation of the difference in the behavior of the two hearts toward lack of oxygen was furnished by Loeb from experiments on the eggs of the two fish in their early stage of cleavage. When oxygen is lacking, substances are formed in the egg of Ctenolabrus which prevent it from segmenting and which dissolve the cell-walls already formed. In the egg of Fundulus this does not take place, but the egg goes on segmenting for many hours.

It seems probable that such substances are formed in the embryo heart as well as in the egg of Ctenolabrus. The ultimate source of energy of vital action is chemical, but to appear as heart-beat this chemical energy (produced by fermentation and oxidation) must be transformed into molecular energy. To obtain this, certain conditions of protoplasm must exist, and these conditions are normally present in the living heart-muscle. But in the absence of oxygen substances are formed by fermentation which may and most likely do produce grave changes in the protoplasm, — such as changes in surface tension, in osmotic pressure, in the spreading phenomena, — and these changes would prevent the transformation of the liberated chemical energy into that molecular energy required for specific physiological activity.

It may be added that the injurious bodies may be formed in two ways. First, they may be the direct products of fermentation. In this case they would be oxidized, if oxygen were present, and thus rendered harmless; but if oxygen were absent, they would retain their original form, and might produce destructive results. Secondly, these bodies may arise from fermentation secondarily, in consequence of the fermentation products reducing material normally present in the cell and thus making it harmful. This action will never occur if sufficient oxygen is supplied. It may further be added that if Hoppe-Seyler's theory of oxidation is correct, oxygen not only furnishes energy by oxidation but plays an equally important rôle in the synthetical action by which complex bodies are built up for undergoing fermentation.

From these considerations it is evident that death by lack of oxygen may be due to one of two causes: (1) Too little reserve material for fermentation. Here the necessary energy is almost directly dependent upon oxidation, and if the oxygen is not supplied regularly, the death of the organ or organism results almost instantly. (2) To the formation of substances that render the transformation of chemical into molecular energy impossible (Ctenolabrus).

We said there seemed to be reasons for believing that high temperature and strong stimuli in general act as does the lack of oxygen. As by stimulation the processes of fermentation are increased, it is evident that if the stimulation be severe and long continued, the fermentation may be so great that the oxygen supply will be insufficient to oxidize all the reducing substances formed. Hence the same conditions would obtain as in lack of oxygen, and death resulting from high temperature could be referred to one of the above two causes.

It was with the object of testing this theory that, at the request of Dr. Loeb, the following experiments were made. Our investigations comprise (1) The effect of high temperature, (2) of lack of oxygen, and (3) of poisons upon the common infusoria, Paramœcium aurelia.

HIGH TEMPERATURE.

The experiments on the effects of high temperature were conducted in the following manner. On a warm stage was placed a glass dish, ten centimetres long, six and a half wide, and three deep, half-filled with water heated to the desired temperature. The Paramœcia were placed in vessels of two different kinds, — the first was a closed dish, holding thirteen to fourteen drops, and admitted no air; the second differed from the first chiefly in that air was admitted. These dishes were so constructed that their contents, having a small bulk and a large area, and being separated from the heated water by thin glass, very speedily acquired the temperature of the surrounding medium. In order to allow for the cooling of the water by the vessel, the water was always heated one degree above the desired temperature, and this was found to balance the cooling quite accurately. The desired temperature of the water was maintained by a regulated gas flame.

(a) **Different temperatures.** — The closed dish (not admitting air) was filled with Paramœcium culture and placed in water of the desired temperature. Column 1 of Table I gives the length of time in minutes required to kill all the infusoria. As the resistance of Paramœcia varies slightly and as it is impossible always to obtain exactly the same conditions, it is not surprising that the series is not perfectly uniform. From these experiments it follows that the duration of life does not decrease in proportion to the rise in temperature, but decreases more rapidly.

Similar experiments were made in the open dish admitting air. The results are found in Column 2 of Table I. From this table it will be seen that the figures corresponding to 36° and upwards are practically the same in Columns 1 and 2. From 36° downwards, however, they vary widely. To what is the difference due?

We think it is because in the one case no air is present (except that absorbed by the water) while in the other, air is admitted. Although the death of Paramœcia at 28° and 30° is not directly due to the lack of oxygen, still this want is an important factor, as is shown by the fact that Paramœcia placed in the open dish under water having room temperature (15°–20° C.) suffered no change in 17 hours, while Paramœcia placed in the closed vessel (not admitting air), at the same temperature and for the same length of time, were nearly all dead. From these experiments we may draw the conclusion that during the short time that Paramœcia live at a temperature of 36° C., the lack of oxygen makes itself felt very little, compared with the effects produced by the high temperature; below 36° C., however, the lack of oxygen is very apparent.

(b) **Heat and concentration of liquid.** — It has been held by some authors that acclimatization to high temperatures is brought about by loss of water from the organism. Davenport and Castle[1] hold this view, for they say: "We are accordingly led to inquire whether the process of acclimatization to higher temperature may not find its cause in the reduction of the amount of water in the chylema spaces, — the organism becoming in this respect more like the spores in which the quantity of free water is reduced to a minimum." It is true that in some physiological phenomena loss of water has the same effect as a lowering of temperature. Loeb[2] found that the sense of heliotropism of Copepods and Polygordius may be changed from negative to positive by lowering the temperature or by the loss of water caused by increasing the concentration of the sea-water. The positive heliotropism can be changed to negative by increasing

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[1] DAVENPORT and CASTLE: Archiv für die Entwickelungsmechanik der Organismen, ii, p. 227.

[2] LOEB: Arch. f. d. ges. Physiol., liv, p. 81.

TABLE I.

Duration of life of Paramœcia at different temperature in open and closed dishes and in different solutions.

Temperature.	Duration of Life in Minutes.				
	1 In Paramœcium culture. Closed dish.	2 In Paramœcium culture. Open dish.	3 In ½ % NaCl solution. Closed dish.	4 In ¼ % NaCl solution. Closed dish.	5 In ⅛ % NaCl solution. Closed dish.
17°C. . . .	1560	....	....	....	....
20 . . .	....	....	185	237	....
22 . . .	....	....	87	165	....
24 . . .	....	....	72	82	144
26 . . .	....	....	36	45	146
28 . . .	115	....	24	39	73
30 . . .	100	....	24	25	72
31 . . .	....	459	....	....	....
32 . . .	....	362	11	16	58
33 . . .	....	268	....	....	....
34 . . .	....	154	9	7; 9	26
35 . . .	48	121	9	....	....
36 . . .	31	38; 31	....	11	17
37 . . .	24	22	7	8	....
38 . . .	13	19	....	....	10
39 . . .	....	14	4	6	....
40 . . .	12; 9; 13	12	2½	....	6
41 . . .	....	....	....	4	....
42 . . .	8	9	....	....	....
43 . . .	....	7	....	....	....
44 . . .	....	4½	....	....	....

the water in the cell (lowering concentration) or by raising the temperature. In some unpublished researches on Fundulus eggs, prior to the publication of Davenport and Castle, Loeb investigated the same question which they discuss. He found, however, that by raising the concentration of the sea-water, the eggs were not able to withstand as high a temperature as when in normal sea-water.

It was with this question in mind that we made a series of experiments, employing sodium chloride solutions, having the strength of 0.5, 0.25, and 0.125 per cent. The experiments were performed by carefully placing thirteen drops of the required sodium chloride solution in the closed vessel and adding one drop of Paramœcium culture. The results are found in Columns 3, 4, and 5 of Table I. It is very evident, from these experiments that with the increase of the concentration of the solution in which the Paramœcia live their power of resistance to high temperature is decreased. Even a $\frac{1}{8}$ per cent sodium chloride solution considerably decreased their duration of life when subjected to high temperatures.

(c) **Acids and alkalies.** — We next made a series of experiments with acids and alkalies to ascertain whether the reaction of the fluid in which the infusoria were heated had any effect upon their power of resistance to high temperature. Isotonic solutions of sodium hydrate, hydrochloric acid, and sodium chloride were made. The dish was nearly filled with a certain per cent solution of alkali, acid, or sodium chloride, a drop of Paramœcium culture was added, and then the dish was heated in the water-bath to the required temperature. Table II gives the results obtained in some experiments with sodium hydrate solution, the Paramœcia being heated to 40° C.

TABLE II.

Temperature 40° *C. — Open dish* (*air admitted*).

Medium.	Duration of life in minutes.	Average in minutes.	Increase over water in minutes.	% Increase over H_2O.
Culture	22; 22; 25; 23; 22	23	....	....
Distilled Water .	(21; 25); 12; 17; 16; 16; 18; 19; 17; 13; 14; 17	17	....	....
$\frac{1}{400}$ % NaOH . .	31; 31	31	14	83
$\frac{1}{800}$ % NaOH . .	(35; 39); 26; 30; 30; 26; 27; 25; 28; 23; 24; 27; 27	28.2	11.2	66
$\frac{1}{1600}$ % NaOH. .	26; 29	27.5	10.5	62

From this table it is evident that a weak sodium hydrate solution ($\frac{1}{400}$–$\frac{1}{1600}$%) increases the power of resistance of Paramœcia to the destructive effects of high temperature. This result was verified by many subsequent experiments. The result of one lot comprising about sixty experiments is represented graphically in Fig. 1. In this figure the degree of concentration of the sodium hydrate and hydrochloric acid solutions is represented by the abscissæ, while the ordinates denote the time in minutes which Paramœcia live in these solutions at 40° C. The full-lined curve represents the length of life at 40° C. in the various concentrations of sodium hydrate, while the broken curve denotes the length of life in hydrochloric acid solutions at the same temperature. The dotted line at 20.5 minutes, running parallel with the axis of abscissa, shows the length of time which Paramœcia live in distilled water at 40° C. As one sees from this figure, the alkali curve between the concentrations of $\frac{1}{400}$% and $\frac{1}{2000}$% is considerably above the straight line which repre-

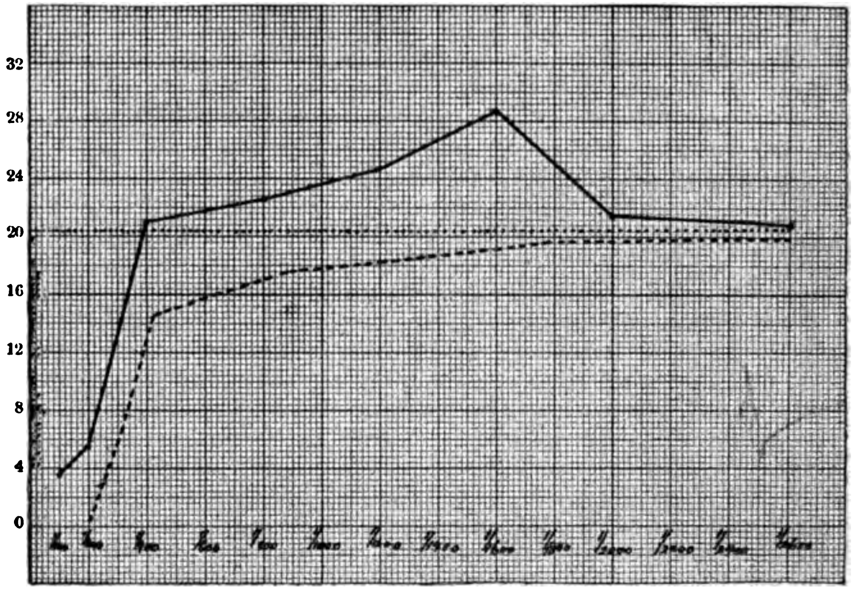

Figure 1. Showing duration of life of Paramœcia in acid (broken line) and alkali (unbroken line) solutions, and in distilled water (dotted line), at 40° C. The abscissæ give the per cent of concentration of the acid and alkali solutions; the ordinates give time in minutes.

sents the duration of life in neutral water. In the acid solutions, however, the duration of life (represented by broken curve) is everywhere below this line, showing the acid solution of all concentrations are injurious if they have any effect at all. If the concentration of the alkali solution is greater than $\frac{1}{400}$% it is decidedly injurious.

The action of a weak alkali solution is still better seen in the following detailed description of two experiments.

Experiment 207.

Temp. 40° *C.* — *Open dish.*

Thirteen drops of distilled water and one drop of Paramœcium culture.
After 10 min. — Movements slower and much rotation.
After 15 min. — Many quiet; others very slow.
After 20 min. — All dead.

Experiment 208.

Temp. 40° *C.* — *Open dish.*

Thirteen drops of $\frac{1}{1600}$ % NaOH and one drop of Paramœcium culture.
After 10 min. — Movements about normal.
After 15 min. — None quiet yet; moving slowly.
After 20 min. — Only a couple quiet; the rest moving slowly.
After 25 min. — A few more at rest; much like at 15 min. in the water medium.
After 28 min. — All dead.

These experiments show that the conditions after 25 minutes in a $\frac{1}{1600}$% NaOH solution at 40° C. correspond very nearly to that after 15 minutes in distilled water at the same temperature.

Now this beneficial effect of the alkali solution might be due (1) to its osmotic pressure, or (2) to its chemical qualities. If the effect is due to osmotic pressure, an indifferent solution, such as sodium chloride, of the same osmotic value ought to have the same effect. From the molecular weights of sodium chloride (58.5) and of sodium hydrate (40) we find that a $\frac{1}{400}$% NaCl and a $\frac{1}{400}$% NaOH solution have the osmotic pressure of 7.239 and 10.602 mm. respectively, not taking into account dissociation. A $\frac{1}{1600}$% solution of sodium chloride and sodium hydrate have the osmotic value of 1.8 and 2.6 respectively. As the increase in duration of life caused by alkaline solutions is found between these two limits, we are chiefly concerned with these percentages, and here the differences of osmotic pressure for the same percentages of sodium hydrate and sodium chloride are so small as to be safely ignored.

Table III gives the averages of the results obtained in the sodium chloride solutions at 40° C.

TABLE III.

Temp. 40° *C.—Open dish (air admitted).*

Percentage of Sodium chloride.	Duration of life in minutes.
0.7	3.5
0.35	8.0
0.1	16.0
0.05	20.5
$\frac{1}{200}$	21.6
$\frac{1}{400}$	19.7
$\frac{1}{1600}$	20.6
0.00	20.0

These experiments prove that a neutral salt solution does not have the power of increasing the resistance of Paramœcia to heat. The peculiar influence of sodium hydrate solution cannot therefore be due to its osmotic pressure, but must be due to chemical influences.

In the same manner in which we tried the effect of alkali we also tried the effect of acid solution. For convenience of comparison we employed hydrochloric acid solutions isotonic with those of sodium hydrate. The osmotic pressure of a $\frac{1}{219}$% hydrochloric acid solution is the same as that of a $\frac{1}{200}$% sodium hydrate solution. A $\frac{1}{1752}$% hydrochloric acid solution corresponds to a $\frac{1}{1600}$% sodium hydrate solution. The results of experiments with hydrochloric acid solutions are represented by the broken curve in Fig. 1. This curve shows that if acids have any effect at all, they have the opposite effect to alkalies, namely, shortening the life of Paramœcia subjected to high temperature. Never does a hydrochloric acid solution increase the power of resistance against high temperature, but even such a weak solution as $\frac{1}{1600}$ per cent decreases the duration of life at 40° C. by about five per cent.

We have thus seen that the peculiar effect of alkali in lengthening the life of Paramœcia exposed to high temperatures (37–40°) is not due to its osmotic pressure. Again we have seen that acids do not possess this peculiar action. From these considerations we may infer that by the action of high temperature a substance is formed in the Paramœcia, which eventually brings about the destruction of the organism. This substance is neutralized by alkali but not by acid. This might lead us still further to suppose that the substance formed is an acid.

LACK OF OXYGEN.

From the theoretical considerations mentioned at the beginning of this paper we concluded that what took place more rapidly at a high temperature might take place more slowly in lack of oxygen, and that therefore alkali would have the same effect as in the case of high temperature. To determine this we made the following series of experiments on lack of oxygen. The air was eliminated by the usual method — replacement by hydrogen. For this purpose the well-known Engelmann gas-chamber was employed. The hydrogen was carefully washed in a potassium permanganate solution, a concentrated solution of potassium hydrate, and distilled water. Two, and in some cases three wash-bottles of the alkali were inserted, so that the current of hydrogen passed three times through a layer of potassium hydrate solution of about nine centimetres thickness. All these precautions were taken in order to remove every trace of acid which might have been introduced with the hydrogen. In the Engelmann chamber was placed a slip of glass to which were attached two rubber rings thirteen millimetres in diameter, and one millimetre high. The cells thus formed were capable of holding seven drops of fluid. In one of the cells were placed six drops of water and one drop of washed Paramœcia, while the other cell held six drops of the alkali solution plus one drop of washed Paramœcia. The current was then let in, and the behavior of the animals observed and the time of death noted.

As the alkalinity of the water in the aquarium may be considerable and as the alkalinity and the concentration of the water may vary from day to day, we thought it well to employ "washed" Paramœcia. It is well known that the Paramœcia in the aquarium collect densely around the edge of the dish, two or three millimetres below the surface. It is therefore easy to collect a great number of the infusoria without taking much of the water of the aquarium. They are then placed in tubes of distilled water. Besides the very slight difference of concentration and alkalinity of the cultures used, the varying strength of the hydrogen current has to be taken in consideration; when the current is strong, the time required will of course be much less than when it is weak. From this it becomes evident that individual experiments are not to be compared with each other. For example, it would be incorrect to compare Experiments 248 and 249.

Experiments 248–254 of Table IV give the results of a series which show that in a weak alkali solution the Paramœcia withstand the lack of oxygen for a longer time than in distilled water. The average increase in these four experiments is 116 per cent. Hence the effect of a weak alkali solution in the case of lack of oxygen is the same as in the case of high temperature; in both the resistance of Paramœcia is increased. To show that here also the influence of the sodium hydrate solution is not due to osmotic pressure, we compared the duration of life in water and in a sodium chloride solution. Experiments 387–396 of Table IV give the results of this inquiry. The increase in the sodium chloride solution is very slight compared with the increase found in Exp. 248–254, where sodium hydrate solutions were employed.

TABLE IV.

Duration of life of Paramœcia subjected to lack of oxygen.

No. of Exp.	Duration of life in minutes.					Increase in NaCl over Water.		Increase in NaOH over H_2O or NaCl.	
	In H_2O.	In NaCl.		In NaOH.					
		% Solution.	Time in min.	% Solution.	Time in min.	In min.	In %.	In min.	In %.
248	15	....	....	$\frac{1}{800}$	40	....	....	25	170
249	65	....	....	"	107	....	....	42	70
252	20	....	....	"	42	....	....	22	110
254	33	....	....	"	71	....	....	38	115
387	50	$\frac{1}{400}$	90	....	....	40	80	....	....
388	32	$\frac{1}{100}$	42	....	....	10	30	....	....
389	62	$\frac{1}{200}$	66	....	....	4	6½	....	....
390	39	$\frac{1}{10}$	42	....	....	3	8	....	....
391	16	$\frac{1}{400}$	13	....	....	−3	−20	....	....
392	32	$\frac{1}{100}$	36	....	....	+4	+32	....	....
393	25	$\frac{1}{10}$	45	....	....	20	80	....	....
394	13	$\frac{1}{700}$	18	....	....	5	40	....	....
395	11	$\frac{1}{700}$	11	....	....	0	0	....	....
396	23	$\frac{1}{700}$	22	....	....	−1	−4½	....	....
380	....	$\frac{1}{100}$	15	$\frac{1}{400}$	95	....	....	80	540
381	....	$\frac{1}{300}$	28	$\frac{1}{400}$	46	....	....	18	65
382	....	$\frac{1}{1600}$	65	$\frac{1}{2000}$	65	....	....	0	0
383	....	$\frac{1}{80}$	11	$\frac{1}{100}$	22	....	....	11	100
384	....	$\frac{1}{160}$	11	$\frac{1}{200}$	39	....	....	28	255

To show this still more clearly we compared the effect of an alkali and a neutral salt solution side by side, the results of which are given in Exp. 380–384, Table IV. The solution of sodium hydrate and sodium chloride here used were almost isotonic. Neglecting dissociation, the osmotic pressure of

$\frac{1}{300}$ per cent NaCl solution = 9.65 mm.; of $\frac{1}{400}$ per cent NaOH = 10.6 mm.
$\frac{1}{100}$ " " " = 18.09 mm.; of $\frac{1}{200}$ " " = 21.20 mm.
$\frac{1}{50}$ " " " = 36.195 mm.; of $\frac{1}{100}$ " " = 42.40 mm.

These last experiments (380–384) show that the average increase of duration of life in a sodium hydrate solution (above $\frac{1}{2000}$ per cent, over that in the sodium chloride solution is 240 per cent. There is therefore no doubt that in lack of oxygen, as in the case of high temperature, the peculiar effect of alkali solutions is not due to osmotic pressure.

It still remains to show the effects of acid. Table V shows the action of hydrochloric acid compared with water.

TABLE V.

No. of Exp.	Duration of life in minutes.			Increase in HCl.	
	In H_2O.	In HCl Solution.			
		% Solution.	In min.	In min.	In %.
385	33	$\frac{1}{438}$	4	−29	−87
386	21	$\frac{1}{438}$	3	−18	−86
398	30	$\frac{1}{876}$	21	− 9	−30
"	24	$\frac{1}{876}$	15	− 9	−37
"	52	$\frac{1}{1752}$	48	− 4	− 8
"	46	$\frac{1}{1752}$	45	− 1	− 2
"	27	$\frac{1}{3504}$	28	+ 1	+ 4
"	34	$\frac{1}{3504}$	32	− 2	− 6

From this table it will be observed that hydrochloric acid solutions, as in the case of high temperature, act unfavorably upon Paramœcia in case of lack of oxygen.

To show the favorable action of a sodium hydrate solution in detail, we append the notes on Experiment 247.

Experiment 247.

Paramœcium aurelia subject to lack of oxygen.

A number of the Paramœcia are placed in distilled water; the others, in a $\frac{1}{100}$ per cent sodium hydrate solution.

1.15 P.M.	The hydrogen introduced into Engelmann chamber.
1.50 P.M.	In the distilled water, some are entirely quiet; some of them are rotating rapidly, with no progressive movements. A few of the smaller are still swimming. In the $\frac{1}{100}$ % NaOH about 80 per cent are still swimming in a most lively fashion. The rest are rotating. Those in the water appear much swollen, being about twice the size of those in the alkali.
2.05 P.M.	Hardly any life left in the water. About half of those in the alkali are still moving about quite lively; a few absolutely quiet; the remaining are rotating.
2.15 P.M.	No more life left in the water. About half of those in the alkali are still moving quite lively.
2.35 P.M.	About one quarter of those in the alkali swim quite briskly.
2.45 P.M.	In the alkali a few are still moving slowly.
	Experiment interrupted.

Thus we have shown that alkali solutions of from $\frac{1}{200}$ to $\frac{1}{2000}$ per cent increase the resistance power of Paramœcia to both high temperature (40° C.) and lack of oxygen. In this respect we have shown the similarity between the physiological effects of both. We have further shown that this action of the alkali is not due to its osmotic pressure; hence it must be due to its chemical composition. Again it has been proven that acids do not increase the power of resistance to these two agents, but that, if at all active, they have a weakening influence. This might lead us one step further in our theory, namely, that the peculiar action of the alkali in these circumstances is due to the fact that it neutralizes a substance, perhaps an acid, formed by strong stimulation and during lack of oxygen.

Poisons.

Through the observations of Claude Bernard it has been proven that if an animal is killed by potassium cyanide the blood in the veins remains arterial, and Geppert proved that potassium cyanide renders the tissues unable to take up oxygen. Such a manner of death is therefore comparable to that brought about directly by lack of oxygen. If this is correct, we ought to be able to demonstrate in the case of poisoning by potassium cyanide, the peculiar action of alkali that we found in the case of lack of oxygen. With this in mind, we made the following series of experiments.

TABLE VI.

One per cent potassium cyanide; effects of alkali.
10 drops of the liquids H_2O, NaOH, HCl, or NaCl +
1 drop of washed Paramœcia plus 1 drop of 1.0% KCN.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H_2O	In NaCl $\frac{1}{200}$%	In NaOH $\frac{1}{200}$%	H_2O	NaCl	H_2O	NaCl	
256	28	11	30	2	17	7	160	Average
257	36	36	61	25	25	70	70	increase in
258	8	7	28	20	21	250	280	min. over
259	12	14	30	18	16	150	115	$H_2O = 13$
260	8	15	26	18	11	225	75	NaCl = 12.6
261	12	12	29	17	17	140	140	In per cent over
262	9	19	23	14	4	155	22	$H_2O = 118$%
263	7	8	13	6	5	90	80	NaCl = 103%
264	11	11	16	5	5	45	45	
265	11	11	16	5	5	45	45	
		0.1%	$\frac{1}{100}$%					Av. increase
282	5	5	21	16	16	320	320	in min. over
283	7	6	21	14	15	200	250	$H_2O = 11.3$
284	8	5	17	9	12	115	240	NaCl = 15.0
285	8	5	21	13	16	165	320	In per cent
286	15	7	23	8	16	55	230	$H_2O = 162$%
287	7	. .	15	8	. .	115	. .	NaCl = 272%

In a small dish, eight or ten drops of distilled water were placed, to which was added one drop of Paramœcia and one drop of the chemical agent to be tested. In a second dish, the water was replaced by a solution of sodium chloride, sodium hydrate, or hydrochloric acid, while the rest remained the same. In most of the experiments three trials were made at once, one in water, one in an

TABLE VII.

One per cent potassium cyanide; effects of acid.
Conditions the same as in Table VI.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaCH
	In H_2O	In HCl $\frac{1}{438}\%$	In NaOH $\frac{1}{200}\%$	H_2O	HCl	H_2O	HCl	
267	12	10	18	6	8	50	80	
268	6	4	11	5	7	85	175	
		$\frac{1}{876}\%$						
269	5	3	7	2	4	40	135	
270	9	8	12	3	4	35	50	
		$\frac{1}{1752}\%$	$\frac{1}{400}\%$					Av. increase in min. over $H_2O = 4$, or 58% $HCl = 4.4$ or 64%
272	5	5	8	3	3	60	60	
273	9	8	13	4	5	45	63	
274	7	7	12	5	5	70	70	
			$\frac{1}{1200}\%$					
275	7	6	7	0	1	0	1½	Av. increase in min. over $H_2O = 2.7$ or 29% $HCl = 3.4$ or 34%
276	8	8	11	3	3	36	36	
277	10	9	15	5	6	50	65	

If 0.7 per cent sodium chloride is used, death occurs almost instantly. If 0.025 per-cent sodium hydrate is used, death occurs in about one minute. The one per cent potassium cyanide has a strong alkaline reaction.

alkali solution, and the third in a sodium chloride solution. The three tests were made side by side for ready comparison, and at exactly the same time.

We first made experiments with potassium cyanide, the results of which are embodied in Tables VI and VII.

TABLE VIII.

One tenth per cent sulphate of Atropine; effects of alkali and acid.

Eight drops of H_2O, NaOH, NaCl, or HCl solutions plus one drop of washed Paramœcia plus two drops of 0.1 per cent Atropine.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H_2O	NaCl $\frac{1}{200}\%$	NaOH $\frac{1}{100}\%$	H_2O	NaCl	H_2O	NaCl	
288	3	3	6	3	3	100	100	Over $H_2O = 5$ min.
289	3	4	10	7	6	235	150	or 124%
290	14	5	17	3	12	21	240	Over NaCl = 7 min.
291	5	5	12	7	7	140	140	or 157%
		$\frac{1}{400}\%$	$\frac{1}{200}\%$					Over $H_2O = 22$ min.
292	8	6	26	18	20	225	330	or 418%
293	4	6	32	28	26	700	430	Over NaCl = 20 min.
294	6	10	26	20	16	330	160	or 307%
		$\frac{1}{800}\%$	$\frac{1}{800}\%$					
295	9	4	11	2	7	22	175	Over $H_2O = 3\frac{1}{2}$ min.
296	4	10	13	9	3	225	30	or 76%
297	12	12	22	10	10	85	85	Over NaCl = 3 min.
298	27	28	20	−7	−8	−25	−30	or 65%
			$\frac{1}{1200}\%$					
299	21	21	22	1	1	5	5	Over $H_2O = 1$ min.
300	13	13	14	1	1	7	7	or 6%
		$\frac{1}{1600}\%$	$\frac{1}{1752}\%$ HCl					
301	2	2	3					
302	4	4	4					
303	5	3	7					
304	25	25	25					
305	4	4	4					
			$\frac{1}{328}\%$ HCl					
306	10		4					
307	11		1					

The 0.1 per cent atropine has no effect upon litmus. A strong solution, however, is decidedly alkaline.

TABLE IX.

Sulphate of strychnine 0.01 per cent; effects of alkali.

Eight drops of H_2O, NaCl, NaOH, plus one drop of washed Paramœcia plus one drop of 0.01 per cent sulphate of strychnine.

No. of Exp.	Duration of life in minutes			Increase in NaOH over H_2O		Average increase of NaOH over H_2O or NaCl
	In H_2O	In NaCl $\frac{1}{100}$%	In NaOH $\frac{1}{100}$%	In min.	In %	
308	5	5	0		−∞	
		$\frac{1}{400}$%	$\frac{1}{200}$%			−73%
310	11	11	4	−7	−65	
311	5	3	1	−4	−80	
312	5	6	1	−4	−80	
313	10	11	3	−7	−70	
		$\frac{1}{1600}$%	$\frac{1}{1200}$%			−40%
317	6	4	4	−2	−35	
318	6	6	4	−2	−35	
319	4	4	2	−2	−50	
		$\frac{1}{3200}$%	$\frac{1}{2000}$%			−9%
322	5	5	4	−1	−20	
323	5	5	5	−0	−0	
324	3	5	3	−0	−0	
325	7	9	6	−1	−15	

From these tables it is apparent that a weak alkali increases the power of resistance against potassium cyanide while a neutral or acid solution does not possess this power. In fact, while the extreme weak alkali solution of $\frac{1}{2000}$ per cent still increases the time twenty-three per cent, a solution of $\frac{1}{1752}$ per cent hydrochloric acid either shortens the time or has no effect whatever. That the action of the sodium hydrate is not due to the osmotic pressure is shown in Experiments 256–265, where the difference of pressure between the sodium chloride and sodium hydrate solutions is but seven millimetres.

From observations made by Rossbach it seems likely that alkaloids may also produce death by rendering the tissues unable to take up oxygen. This led us to test the action of acids and alkalies in case of poisoning by atropine, strychnine, etc. The results obtained with atropine poisoning are embodied in Table VIII.

In the experiments with atropine, the beneficial action of sodium hydrate is still better shown than in the case of potassium cyanide. When it is remembered that the alkaloid is already alkaline in its reaction, it is evident that this action of sodium hydrate is not due to the neutralization of the alkaloid. That this peculiar action of alkali is not due to a general stimulating effect or the neutralization of the poison, the following experiments on strychnine (308–329) prove conclusively.

TABLE X.

Sulphate of strychnine 0.01 per cent; effects of acid.
Conditions the same as in Table IX.

No. of Exp.	Duration of life in minutes.			Increase in NaCl.		Average Increase.
	In H_2O.	In NaCl 0.1 %.	In HCl $\frac{1}{438}$ %.	In min.	In %.	
326	5	18	0.0	13	260	Of 0.1 % NaCl over $H_2O = 8.2$ min. or 144 %.
			$\frac{1}{1752}$ %			
327	8	16	7	8	100	
328	11	18	11	7	160	
329	9	14	9	5	55	

Sulphate of strychnine 0.01 per cent has no effect upon the litmus, but a stronger solution is decidedly acid in reaction. If 0.7 per cent sodium chloride is used, the Paramœcia perish in one or two minutes.

From these experiments it is apparent that a sodium hydrate solution, instead of exercising a beneficial influence, is highly destructive. This is so radically different from the trend of all the preceding experiments, that I doubted the purity of the solution. But on testing, I found that the Paramœcia in a $\frac{1}{200}$ per cent sodium hydrate solution lived for more than three hours.

Considering that the strychnine is acid in reaction, while the potassium cyanide and atropine solutions are alkaline, we would *a priori* expect that an alkali ought to be more beneficial when added to a strychnine solution than to the others. Instead we found the opposite true. Most likely the chemical action of the strychnine in the cell is different from that of potassium cyanide or atropine. This corresponds with the difference in the physiological effects of these poisons. But even in some alkaloids having an alkaline reaction, the addition of a weak alkali acts unfavorably, as is shown in Experiments 353-358.

TABLE XI. — VERATRINE.

Thirty-five mg. of veratrine boiled in 10 c.c. of water and filtered.
Eight drops of H_2O, HCl, NaCl, or NaOH solutions plus one drop of washed Paramœcia plus two drops of veratrine.

No. of Exp.	Duration of life in minutes.		Decrease in NaOH (in min.)
	In H_2O.	In NaOH	
		$\frac{1}{200}$ %.	
353	26	2	24
354	40	2	38
355	28	$1\frac{1}{2}$	$26\frac{1}{2}$
		$\frac{1}{400}$ %	
356	41	10	31
357	18	15	3
		$\frac{1}{1752}$ % HCl	
358	36	34	

If a $\frac{1}{400}$ % HCl solution is used, the Paramœcia are destroyed almost instantly. The reaction of this veratrine solution is slightly alkaline.

These experiments show that in case of veratrine poisioning, alkali does not increase the duration of life of Paramœcia, but shortens it considerably. Experiments on other poisons are in progress.

When we review the poisons thus far experimented upon we find that their chemical reaction, whether acid or alkaline, does not determine whether the addition of an alkali renders the organism more resistant to its actions or not. The effects of strychnine, although it has an acid reaction, are not counteracted by alkalies. Although potassium cyanide and atropine are alkaline, yet their effects are reduced very markedly by sodium hydrate solutions. It may be further remarked that in no case of poisoning has the addition of an acid increased the resistance of Paramœcia against the poison. As in the case of heat and lack of oxygen, acid, if active at all, decreases the duration of life of the infusoria.

THEORETICAL REMARKS.

Some authors[1] have attributed death caused by a temperature between 35° and 40° C. to a process of coagulation. Others suppose that coagulation is not the direct cause of death, but that other besides physical causes may be present. Among those who sought to attribute death produced by a temperature of 35° to 40° C. to chemical, and not only to physical causes, may be mentioned Sachs, Engelmann, and Rossbach.

Finding that the life of plants is destroyed by a temperature much below that of coagulation, Sachs[2] says, "that the coagulation of the protoplasm is not the only point which determines death at high temperature. . . . So long as outside influences do not exceed a certain amount of force, they are not able to subdue the molecular force [in a cell] which binds together the inner organic structure. But if a force, *e. g.* heat, reaches an intensity which exceeds the molecular force, the molecules are torn from their normal position, and the inner structure which serves as the carrier of life is destroyed without any real changes in the outward form." Roth[3] extended our knowledge of death at high temperature by showing that alkali solutions are able to revive cilia in heat rigor. Two years later Engelmann confirmed this fact. Speaking of this relation between heat and alkalies he says: "It seems to me that the increase of mechanical activity of the cell and the acceleration of the movements produced by heating, are mostly due to the increase in the physiological metabolism (Stoffumsatz). According to our view, in the cells whose movements are increased by heating, the formation of acids is increased. This increased acid formation can, in part, be the cause of the rigor which sets in at 40° C."[1] Rossbach[2] thinks that we cannot in general consider coagulation as the cause of the cessation of movements at high temperature.

---

[1] See KÜHNE: Untersuchungen über das Protoplasma und die Contractilität, Leipzig, 1864.

[2] SACHS, J.: Flora, 1864, xlvii, pp. 5, 24, 33, 65.

[3] ROTH, M.: Virchow's Archiv f. pathol. Anatomie, 1866, xxxvii, p. 184.

Our conception agrees somewhat with that of Engelmann, as do also, to a certain extent, the facts found.

Our object, as stated in the introduction, was to endeavor to establish the similarity between the effects of high temperature and lack of oxygen. Heat, below the point of coagulation, by increasing fermentation, sets free chemical energy, which can be transformed into molecular energy, appearing in such vital phenomena as ciliary motion, contracting of vacuoles, etc.

But we have above supposed that if the stimulation be very strong and of long duration, the oxidation would be insufficient to oxidize all the reducing products formed by fermentation, and that these products would act upon the contents of the cell in such a way as to render the change of chemical into molecular energy impossible, thereby producing death. *A priori*, it hardly seems likely that Paramœcia in an indifferent solution should die in from thirty to sixty minutes because of lack of energy caused by want of oxygen. But if destructive substances be formed, it is easily seen how a sudden death might occur, when we bear in mind the rapid death produced by potassium cyanide and alkaloids. Now from the fact that alkali increases the resistance of Paramœcia to both lack of oxygen and high temperatures, we surmise that heat, below the point of coagulation, brings about death in the same manner as lack of oxygen. It may be supposed that the harmful substances, formed by metabolism at high temperature and in lack of oxygen can in some way be rendered inactive, and in this we believe lies the beneficial action of alkali. In some way or other, either by neutralizing their effects or by attacking the substances themselves, alkali delays death. Seeing they are rendered harmless by alkalies and not by acids, it seems possible that these products formed by fermentation are acids. This also agrees with the experiments of Gaule,[1] who made the important discovery that a weak sodium hydrate solution (1 : 20,000) revived the excised frog heart come to rest in a saline solution. He showed also that such an alkaline solution after being repeatedly driven through the heart underwent a loss in alkalinity, due, no doubt, to an acid formed in the heart muscle.

---

[1] ENGELMANN, T. W.: Ueber die Flimmerbewegung, Leipzig, 1868. See also ENGELMANN: Centralblatt für die medicinische Wissenchaften, 1867, p. 657.

[2] ROSSBACH, J.: Arbeiten aus der zoologischen und zootomischen Institut in Würzburg, 1874, i, p. 9.

As to the action of potassium cyanide and atropine, it is to be borne in mind that we do not regard these substances as stimuli, as has been well shown by Rossbach in the paper above referred to. This author also pointed out the similarity of action between lack of oxygen (by hydrogen atmosphere) and alkaloids, and he thinks that we may consider the cessation of oxidation of the protoplasm as the ultimate action of alkaloids. Supporting this view, Dr. Budgett[2] has shown the striking resemblance between the morphological changes produced in case of lack of oxygen and by alkaloid poisoning upon Paramœcia.

If it is true that these poisons kill by diminishing the power of oxidation, while fermentation continues, reducing substances will be formed, and these substances will have the effects already described. Alkali will therefore have the same influence as in lack of oxygen and high temperature. It may be added that there remains the possibility of the sodium hydrate having some chemical reaction upon the potassium cyanide and atropine, but as the percentage of alkali used is so very small, this becomes highly improbable.

SUMMARY.

Alkalies in very small percentage ($\frac{1}{400}$ – $\frac{1}{2000}$ per cent) increase the resistance of Paramœcia to heat (36–40°C.), to the lack of oxygen, and to the destructive action of potassium cyanide and atropine. Acids (hydrochloric), on the other hand, never increase, but when at all active, always decrease the power of resistance to the agents named.

These facts we try to explain in that the lack of oxygen (produced by hydrogen atmosphere or by poisons preventing oxidation) and high temperature cause the formation of certain substances which render the transformation of chemical into molecular energy impossible, thereby producing death. These substances are in some way antagonized by alkalies, but not by acids.

---

[1] GAULE, J.: Archiv für Physiologie, 1878, p. 291.

[2] BUDGETT: This journal, 1898, i, p. 98.

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INFARCTION IN THE HEART.

By W. BAUMGARTEN.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

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I. REMARKS ON THE PRESENT STATE OF OPINION CONCERNING INFARCTION IN THE HEART.

THE closure of a large coronary artery is frequently a cause of sudden death, and if this peril be escaped, necrosis of the portion of the heart nourished by the closed artery may lead to dilatation, aneurism, or rupture, or may be followed by a scar. Yet closure is not always mortal. At times the pathologist finds the heart wall healthy in the area once supplied by an artery evidently long diseased and probably long occluded. The necrosis which should have followed is not there. Frequently indeed the arteries are so narrow as to be obviously incapable of feeding their vascular areas with the full streams which the laborious heart demands. Many questions are raised by these extraordinary phenomena. Whence comes the blood that keeps the part in life after its normal arterial supply has failed? Do the coronary arteries anastomose? Is each portion of the heart wall traversed by branches from several arteries, or does each artery feed a district exclusively its own? Of what assistance are the coronary veins and the vessels of Thebesius? How long does the ischæmic part remain contractile? These and many similar problems confront us.

One of these problems has been much studied. Attention has long been paid to the possibility of anastomosis of the coronary arteries. The description of these vessels accepted for many years declared that the right and left coronary arteries anastomose freely and that this anastomosis is particularly rich at two points, — the junction of the posterior interventricular with the auriculo-ventricular grooves, and the junction of the anterior and posterior ventricular grooves at the apex of the heart. This view is said to have been first expressed by Ruysch.[1] It was upheld by the anatomists of the early part of the eighteenth century. In the middle of that century the accuracy of the description began to be questioned. The true condition was stated by Senac. The main coronary arteries anastomose only through the smallest arteries and through capillaries, and the communication is so slight that the closure of an artery puts a stop to the circulation in the part which it supplied. A collateral supply through communicating branches is not established. Great names support this description. Thus Henle[2] writes that Hyrtl's[3] statement that no anastomosis takes place between the larger branches of the right and left arteria coronaria is easily confirmed by injecting the two arteries with injection masses of different colors (p. 87). Moreover, observations have been made on the living animal. Cohnheim and v. Schulthess-Rechberg[4] severed the coronary artery in dogs and observed that little or no blood escaped from the distal end of the severed trunk. Infarctions have been produced experimentally by Kolster[5] and by Porter.[6] They are the invariable consequence of the ligation of coronary arteries in the dog. Notwithstanding these and other concordant observations, occasional authors continue to speak for rich anastomosis, and the erroneous idea of the "two circles" still exerts an unfortunate influence.

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[1] RUYSCH: quoted by DRAGNEFF: Bibliographie anatomique, 1896, iv, p. 114.

[2] HENLE: Handbuch der Anatomie des Menschen, Gefässlehre, 1868, pp. 85–87.

[3] HYRTL: Lehrbuch der Anatomie des Menschen, 1889, pp. 1025–1027.

[4] COHNHEIM and v. SCHULTHESS-RECHBERG: Archiv f. pathol. Anatomie, 1881, lxxxv, p. 510. Also PORTER: This journal, 1898, i, p. 155.

[5] KOLSTER: Skandinavisches Archiv für Physiologie, 1893, iv, p. 1.

[6] PORTER: Archiv f. d. ges. Physiol., 1893, lv, p. 366.

The causes of this regrettable conflict over a question of much practical importance are to be found in an inexact conception of the nature of "terminal" arteries, and in imperfect knowledge of the ways in which the heart muscle in the area of an occluded artery may be saved from starvation. It has too often been assumed that the coronary arteries are not terminal because fine injections into one artery can be made to pass into other arteries.[1] It is just this assumption that reveals the obscurity in many minds. It is quite true that fine injections can be made to pass from one coronary artery into another. But this proves nothing except that the arteries communicate, and communication has never been denied. It is the kind of communication which is important. The idea of terminal arteries is physiological, not anatomical. They differ from other arteries in that the peripheral resistance in the anastomosing vessels is too high to be overcome by the blood pressure in any of the arteries of which the communicating vessels are branches. The rapid closure of the terminal artery cuts off the nutrition of its capillary area because sufficient blood for the life of the area cannot be sent through the communicating vessels on account of the high resistance in them. The resistance in the communicating vessels and not merely their size is the determining factor. Artificial injections on dead or "surviving" hearts cannot imitate the intricate complex which determines the resistance to the passage of normal blood through normal vessels.

It has been thought by some that an adequate arterial anastomosis is the only possible explanation of the survival of areas the arteries of which are occluded. Sometimes an anatomosis is found. Dragneff,[2] in a recent study of a series of sixteen human hearts, found an anastomosis in two cases, that is, in thirteen per cent. When the occluded artery happens to be one of the anastomosing exceptions, the explanation of the survival of its area is easy. These rare coincidences the pathologist often has generalized. Where no infarction occurs and no anastomosis is found, it is often thought that arterial anastomosis nevertheless is present, but has been overlooked. Another explanation of survival is furnished by the recent investigation of Pratt,[3] who has demonstrated that the mammalian heart will beat for a very long time when fed at a pressure of an inch or two of blood through the veins of Thebesius or the coronary veins. Evidently this method of nutrition may be of the first importance to the life of parts, the arteries of which are not too rapidly closed. Further, it has been demonstrated that in some situations purely capillary anastomosis may suffice for an adequate collateral circulation, and under certain conditions, as when the circulation is vigorous and the occlusion brought about slowly, this possibly may be true of the heart. Finally, it should be remarked also that we are yet in ignorance of the rôle which may be taken by lymphatics in the rescue of areas the arteries of which are very slowly occluded.

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[1] MICHAELIS: Zeitschrift für klin. Medicin, 1894, xxiv, p. 270.

[2] DRAGNEFF: Bibliographie anatomique, 1896, iv, p. 111.

[3] PRATT: This journal 1898, i, p. 86.

It is evident from these reflections that neither the passage of fine injections from one coronary artery to another, nor the occasional presence of arterial anastomosis, nor the rare survival of parts, the supplying artery of which is wholly or almost wholly closed, weighs in the balance against the facts that the distal stumps of severed coronary arteries bleed little or not at all,[1] that in the human heart the areas of closed arteries are almost always found to be infarcted, and that ligation of the coronary arteries in the dog's heart has thus far without exception been followed by the death of the part supplied. The coronary arteries are certainly terminal, with rare exceptions. The rarity of these exceptions should not be challenged by incautious statisticians. The fact that anastomosis may be present in thirteen per cent of the hearts examined, as in Dragneff's series, is true enough so far as the number of hearts is concerned, but has little real value. The chance of survival after closure depends on the percentage of arteries which anastomose — not on the percentage of hearts in which the anastomosis takes place. There are very many sufficiently large arteries in every heart. The chance that the artery occluded shall be one of those which happen to anastomose is extraordinarily small, and the weight to be attached to these rare cases in deciding the terminal nature of the arteries of the heart is therefore extraordinarily slight.

It follows further from the preceding considerations that the injection method is not well adapted to the study of the distribution of the coronary arteries. It is exposed to both objective and subjective errors. Objective errors are unavoidable. The distribution of the blood is determined during life by the peripheral resistance, and the peripheral resistance of the organ during injection is not that of the normal organ. The normal resistance depends on the inimitable arterial tonus. It depends further on the condition of the tunica intima, a highly sensitive structure, readily altered post mortem. Finally, it is dependent on the composition of the blood. Very slight changes in the blood are known to affect the flow through the vessels. The masses used for injection are widely different from the blood. The normal circulation through capillaries and the vessels next them in size is a function of the blood pressure in the smallest arteries. The exact amount of this pressure is not known, and probably never can be known, for it depends on a great number of interacting intricately balanced factors. Subjective errors are equally unavoidable. The observer who finds a communication must judge whether it was large enough in the normal state to so diminish the peripheral resistance that the normal blood pressure in the next larger artery could drive blood through it — obviously an impossible judgment. In short, the distribution of a terminal artery is a physiological question. The anatomist cannot determine it with certainty. The area which the terminal artery can keep alive is really the area which it supplies. This area is circumscribed by the points at which the resistance in the branches communicating between the artery and its neighbors becomes too great to be overcome by the blood pressure in those branches. The distribution of the coronary arteries should be mapped out by means of the infarctions which follow the ligation when the animal is kept alive, and by observations on human infarction.

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[1] A few drops of blood may be squeezed out by the compression of the intramural vessels in the systole of the ventricle (PORTER: This journal, 1898, i, p. 155).

It was for the purpose of mapping out the distribution of the coronary arteries by the infarction method that the present investigation was begun. It was resolved to study the problem also by means of injection with thin masses containing substances opaque to the Röntgen rays, — a method which avoids destructive dissections and corrosions, and which renders visible the vessels buried in the substance of the heart as well as those upon the surface. The overlapping or interlocking of areas, the character and especially the completeness of the infarction, the time at which the infarcted area ceases to be contractile, and the effect of infarction upon the sounds of the heart, were also studied.

The animals used were the cat and dog.

II. THE ANATOMICAL DISTRIBUTION OF THE CORONARY ARTERIES.

Henle's[1] description of the course of the coronary arteries in the human heart is as follows: "The two coronary arteries, of about equal diameter, arise from the aorta in the right and left sinuses of Valsalva, and proceed downward and outward for a few millimetres on the surface of the heart, between the auricular appendix and the conus arteriosus; each supplies a small branch to the conus arteriosus and the auricular septum. The right coronary artery turns to the right at the auriculo-ventricular groove, and passes between the right auricle and ventricle to the posterior surface of the heart, where its terminal transverse branch usually continues across the posterior interventricular furrow, and is distributed to the right half of the posterior wall of the left ventricle. A series of small branches is given off to the right auricle, and large branches are sent downward over the surface of the right ventricle; one of these descends at the right border of the heart, and another in the posterior interventricular groove to the apex. The left coronary artery divides at the auriculo-ventricular groove into two branches, one descending in or near the anterior interventricular groove, while the other turns to the left to continue between the left auricle and ventricle to the posterior surface of the left ventricle. The descending ramus distributes branches toward the left to the anterior wall of the left ventricle and several branches inward to the interventricular septum. The transverse branch supplies the left auricle and the left border of the left ventricle, its size and distribution varying with the size and distribution of the transverse branch of the right coronary artery. The larger of these usually supplies a branch to the upper posterior half of the interventricular septum."

In order to determine accurately the differences in detail between the distribution of the coronary arteries in man and in the cat and dog, injections of the coronary arteries were made with a mass consisting of equal parts of starch and bismuth subnitrate, rubbed up with water to the consistency of thin cream. The heart was then examined under the Röntgen rays with the fluorescope and finally radiographed by laying it, whole or appropriately divided, on dry plates specially prepared and exposing it a sufficient time to the Röntgen rays. In successful injections this procedure gave the distribution of very small arteries (0.2 to 0.1 millimetre in diameter). It has already been pointed out that this method avoids destructive dissections and corrosions — the tissues remain intact.

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[1] HENLE: Gefässlehre, 1868, p. 85.

The coronary arteries in the cat's heart. — The two main coronary arteries of the cat's heart arise from the aorta in the manner already described for the human heart. The left is always the larger of the two, and has a wider distribution. It supplies the whole of the left ventricle and auricle, the greater part of the interventricular septum, and a portion of the lower posterior wall of the right ventricle, upon which it often encroaches further. From the aorta it passes downward to the left and bifurcates just before reaching the auriculo-ventricular groove into two branches of unequal size. The smaller branch (ramus descendens) passes obliquely downward in the anterior interventricular groove to the apex of the heart. In its course it gives off to the left four or five large branches which ramify over the surface of the anterior wall of the left ventricle. Two or three small branches are given off to the right, but do not pass on to the right ventricle. Of the deep branches the most important is the artery of the septum, which arises about one millimetre below the bifurcation of the main trunk and passes downward and backward into the ventricular septum, at first close to the upper border of the septum, then curving downward along the posterior margin to its lower angle. The other deep branches of the descendens are small, and supply the anterior portion of the septum towards the apex.

The larger branch of the left coronary artery (ramus circumflexus) curves backward in the auriculo-ventricular groove around the left border of the heart. Arriving at the posterior interventricular groove, it bends downward at a right angle and proceeds along the groove to the apex of the heart. The main stem rarely encroaches upon the right ventricle. Near the apex, however, and sometimes nearer the base, superficial branches are given to the right ventricle. A large branch — at the left border of the heart — and many smaller branches are given to the left ventricle. The left auricle receives two large and several smaller branches. One of the two larger branches arises close to the origin of the circumflex, proceeds upward over the anterior surface of the auricle between the auricular appendix and the aorta, and ramifies over the superior surface of the auricle; the other is given off on the posterior surface and passes upward to the left of the pulmonary veins, where it divides, one branch going to the appendix and upper surface of the auricle, the other to the right

— sometimes reaching the right auricle. In the posterior interventricular groove, the descending stem gives off a number of branches to the upper and posterior margins of the septum.

The right coronary artery passes almost horizontally through a mass of fat into the auriculo-ventricular groove, and winds backward around the right border of the heart to or near the posterior interventricular groove. Here the artery, now very small, often turns abruptly and passes downward and forward for a short distance in the wall of the right ventricle. At the right border of the heart it gives off a large vessel which supplies the upper three fourths or more of the right ventricle. Near the aorta a short branch passes to the conus arteriosus. The auricles receive branches similar to the auricular vessels derived from the ramus circumflexus of the left coronary artery; the posterior one of these passes to the right side of the inferior vena cava and sends a branch toward the left auricle, between the two cavæ. The right coronary artery also supplies the upper part (basal portion) of the septum, the size and number of the vessels depending on the relative size of the ramus circumflexus sinister. No artery was found in the interauricular septum.

The coronary arteries in the dog's heart. — The distribution of the coronary arteries in the dog and cat differ (1) in that in the dog the artery of the septum usually arises independently from the left coronary artery, close to the origin of the ramus descendens and ramus circumflexus. The arteria septi passes closer to the anterior margin of the septum than in the cat, and supplies less of the upper posterior portion, which is left mainly to branches of the circumflexus. (2) The descendens gives off just below the middle of its course an important branch, which passes obliquely to the left and supplies the lower third of the left ventricle. (3) The terminal portion of the descendens usually supplies more of the apex than in the cat. (4) The branches passing from the descendens to the right are larger; they encroach somewhat upon the surface of the right ventricle. The deep branches are few and small.

The anastomosis of the coronary arteries studied with the Röntgen rays. — The question of anastomosis receives considerable light from the method of examination with the Röntgen rays, which obviates the disturbance and destruction of small vessels which must occur in dissections or corrosions. Eight cat and eight dog hearts were injected with the starch-bismuth mass and examined with the fluorescope and by radiography. Anastomoses between vessels 0.1 millimetre in diameter were not infrequent, though not numerous. Vessels smaller than 0.1 millimetre were not injected by the mass employed. Only in one case was there found an anastomosis which could be supposed effective. This instance of free communication occurred in a dog heart in the posterior wall of the right auricle, between posterior auricular branches about 0.7 millimetre in diameter derived from the right coronary and circumflex arteries. The right coronary artery in this dog was completely injected from the circumflex branch of the left. This case is peculiarly instructive for the reason that although the injection mass passed through the anastomosis with great ease, the anastomosis had failed to re-establish the circulation through the right coronary artery, which had been ligated thirty-six hours before, as will be detailed in a subsequent paragraph. It may be well to note that the single anastomosis which Cohnheim found in a large number of injections of the dog's heart, as well as one of the two which Dragneff found in his injections of sixteen human hearts, were also between the posterior auricular branches of the right coronary and circumflex arteries.

III. THE PHYSIOLOGICAL DISTRIBUTION OF THE CORONARY ARTERIES.

I purpose now to determine the areas within which the arteries of the heart perform their physiological function, — the areas which they normally supply with blood, and which accordingly depend on them for normal nutrition. Evidence has been presented to show that the coronary arteries are beyond question terminal. The ligation of a terminal artery deprives the part to which it is distributed of blood, gives over its charge to coagulation necrosis, and thus maps out the area in which its physiological function is performed.

The arteries ligated were the circumflex, descending, and septal branches of the left coronary artery, and the main trunk of the right coronary artery. In the cat the circumflex was ligated three times, the ramus descendens eight times, and the right coronary artery once. In the dog, the circumflex was ligated once, the descendens four times, the arteria septi three times, and the right coronary artery once.

The infarcts produced by the ligation of the right coronary or of one of the main branches of the left coronary artery in different animals of the same species are in the main identical in extent.

Method of operating. — All the operations were performed with strict asepsis. In operating on cats, the animal was anæsthetized with ether, tracheotomized, and an incision extending from the second to the sixth rib of the left side made through the skin and the pectoral muscles, close to the margin of the sternum. To prevent bleeding from the intercostal arteries sterilized ligatures were passed around the third, fourth, and fifth costal cartilages near the sternum and again near the outer margin of the wound, the ends being left long, so that they might be used later to draw the severed ends of the cartilages together. The three cartilages were now divided an inch to the left of the sternum. With the opening of the thorax, artificial respiration was begun. The parietal pericardium was freed from fat sufficiently to permit an incision one to two centimetres long, parallel to the left margin of the conus arteriosus. The visceral pericardium was then torn slightly with forceps near the course of the artery to be ligated, the artery laid bare with a blunt seeker, and a ligature passed around it with a small curved threaded hook bent at right angles to the shaft. No attempt was made to close the incision in the pericardium. The ends of the divided costal cartilages were brought together by means of the long ends of the ligatures which had been passed around them. The external wound was sewed up and dressed with a layer of corrosive sublimate gauze and a thick pad of cotton. After the animal had begun independent respiration, the tracheal cannula was removed and the wound in the neck closed. Finally, the chest and neck were thoroughly bandaged.

The procedure in the case of dogs was not essentially different. Dogs received from one to three centigrams of morphine sulphate half an hour before etherization. The dose was repeated from two to four hours afterward to prevent restlessness. The operation outlined above was modified in that the second rib was also divided, and incisions two to three inches in length were made in the first and fifth left intercostal spaces so that the wall of the thorax could be drawn aside and the opening into the chest enlarged.

In several dogs the arteria septi was ligated.[1] As the difficulty of this operation has been commented on by Professor Kronecker[2] it may be stated that special precautions are needed to secure its success. There should be four assistants, one to blow air into the lungs through a two-necked Wolff's bottle containing ether, a second to hold the lips of the wound apart with heavy retractors, a third to assist with the instruments, and a fourth to hold an electric lamp in such a way that the operator can catch the light upon a frontal mirror of the sort used by laryngoscopists. This mirror is nearly indispensable. It should be turned up against the forehead so that both eyes may be uncovered. With this light the slight differences in color between muscle fibres and small arteries are much more easily made out. The heart is laid bare as described above. The edges of the pericardium are sewed to the chest wall with two stitches on each side — thus raising the heart. The operator seizes with dissecting forceps the fat and connective tissue at the base of the aorta near the origin of the left coronary artery, and with a second dissecting forceps removes the tissues over the junction of the ramus circumflexus and ramus descendens. A blunt flattened seeker is then employed to press back the muscle fibres and other tissues about the junction, burrowing under until the arteria septi comes into view. If the latter arise from the right anterior aspect of the main trunk or the ramus descendens, it may be found easily; if from the left posterior aspect, the search will be long and trying; in such case the ramus descendens may be raised for a few seconds — not longer — upon a flattened bent hook. Ligation is accomplished by passing a threaded slightly curved aneurism hook beneath the ramus septi, releasing the handle, grasping the end of the thread with dissecting forceps and drawing back the aneurism hook. In the preparation of the artery no blood whatever must be lost; a few drops will so stain the tissues as to render success very doubtful. The assistants must be thoroughly trained, and the operation must be done rapidly and without a flaw. It is particularly necessary that the bellows be managed skilfully.

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[1] These operations were done by Dr. Porter.

[2] Kronecker: Zeitschrift für Biologie, 1896, xxxiv, p. 554.

The after treatment of the animal is nearly as important to recovery as the manner of operating. Cats should be placed in a small box upon clean straw and kept warm until the immediate effects of the operation have passed away. Dogs should be laid on the side upon the dog-board and supported on comfortable cushions the covers of which are made of glazed stuff that can be wiped clean. The fore-limbs are held near the board by one short tether, the hind limbs by another. A leather strap passes over the chest, and a second strap over the neck, both too loose to press upon the dog, but tight enough to prevent his slipping off the board or raising the head. These precautions lessen the risk of heart failure. Morphia should be given in very small quantities to prevent restlessness during the first day. The care of the animal should be that received by a serious case in a hospital ward.

Infarcts in the cat's heart. — The extent of the infarct following ligation of the ramus descendens in eight cats was almost the same in each case. Variations were far slighter than in the infarcts following ligation of either of the other arteries. The infarct of the descendens includes the whole of the anterior wall of the left ventricle from the anterior interventricular furrow to the left border of the heart, except a small triangle in the upper left corner of the surface. It also includes the anterior portion of the apex of the heart, the anterior two thirds of the septum, and the anterior papillary muscle. The descendens area thus forms a quadrilateral, one angle of which is at the point of ligation. The infarct does not extend above the auriculo-ventricular groove, nor does it involve the whole apex of the ventricle, nor the posterior third and upper border of the septum. On section, the infarct is seen to include the whole thickness of the anterior wall of the left ventricle and the left papillary muscle. But in the septum only the left half, the side turned towards the left ventricle, is infarcted.

The infarct following the ligation of the circumflex artery involves a much wider area. It extends over the posterior wall of the left ventricle and auricle, from the apex of the heart to the point of entrance of the pulmonary veins into the left auricle. At the upper end of the posterior interventricular groove a small triangular space on the left ventricle remains untouched. The posterior papillary muscle, the posterior third of the septum, and a variable extent of the surface of the right ventricle are also infarcted. In the left auricle, the posterior wall up to the superior border, and the posterior border of the appendix are infarcted. The pulmonary veins show no evidence of infarction. On section, it is observed that the posterior wall of the left ventricle and auricle, and the posterior papillary muscle, are infarcted through their entire thickness, whereas in the septum the posterior third is usually involved only on the side facing the left ventricle, and in the case of the right ventricle only the pericardial surface is attacked.

The infarct produced by ligation of the right coronary artery occupies the territory between the descendens and the circumflex area, namely, the whole thickness of the greater part of the wall of the right ventricle, the whole thickness of the posterior wall of the right auricle, and the posterior portion of its appendix, and finally the right ventricular aspect of the posterior third of the septum. The circumflex and right coronary areas commonly overlap to a variable extent on the posterior surface of the right ventricle; in these cases, the right coronary infarct involves the endocardial portion of the wall of the ventricle, while the circumflex infarct occupies the pericardial portion, as has already been mentioned.

In these several ligations the auricular appendices were not completely infarcted because the nutrient branches to their anterior wall arise close to the origin of the main trunks and were therefore not included in the ligatures.

The inferior vena cava remained uninvolved.

Infarcts in the dog's heart. — In the heart of the dog the areas of infarction following ligation of the circumflex and the right coronary arteries are similar to those of the same arteries in the cat. The infarct of the circumflex exhibits some variation; in two cases it involved the whole apex of the heart. The chief points of difference between the two animals relate to the descending branch of the left coronary artery. In the dog, the artery of the septum is given off independently of the descendens, or in rare instances very close to its origin, so that the arteria septi is not included in the ligation of the descendens. In the dog the descendens infarction is therefore limited chiefly to the anterior wall of the left ventricle and the inferior (apical) anterior portion of the septum. To a slight extent, but relatively further than in the cat's heart, it encroaches on the right ventricle. The infarct produced by ligation of the septal artery is triangular in shape, with the apex of the triangle near the ligature. Whether the auricular septum was involved in this infarct was not observed.

The infarction in a dog in which the descendens was ligated by W. T. Porter in 1893[1] will be of interest here. This dog lived four days. Post mortem it was found that the artery was tied six millimetres from its origin. A thrombus filled the artery on the proximal side of the ligature. The interventricular septum, which was about 45 mm. broad and 6 mm. thick, contained a white wedge-shaped infarct. Seven millimetres below the ligature (apical direction) the infarct measured 13 mm., and 25 mm. below the ligature 23 mm. from posterior to anterior margin. A similar wedge occupied the anterior wall of the left ventricle, extending outwards from the interventricular furrow. The apex of this part of the infarct lay near the ligature. Thirty millimetres below the ligature (apical direction) its breadth was nearly 30 mm. The infarct was plainly visible on the inner surface of the ventricle. It comprised the whole thickness of the ventricular wall.

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[1] PORTER: Arch. f. d. ges. Physiol., 1893, lv, p. 367.

One case in my series of infarcts in the dog's heart deserves particular attention. In this case the right coronary artery was ligated, and the animal was killed thirty-six hours later. As the infarct did not seem to be well marked on the pericardial surface, the circumflex artery was injected in order to determine the existence of an anastomosis. This injection succeeded without difficulty in filling the trunk and all the ramifications of the right coronary artery, and demonstrated a large, well-defined anastomosis 0.7 mm. in diameter in the posterior auricular wall. The infarct proved to be complete, the macroscopical appearance being corroborated by microscopical examination. No better illustration could be desired of the extent to which the arterial tonus controls the peripheral resistance and thus determines within wide limits whether a communicating branch shall during life be wide or narrow — an open or a closed gate for collateral circulation.

Variations in the areas of infarction. — Variations in the boundaries of infarcts are particularly marked in the infarcts of the circumflex and right coronary arteries. The descendens shows a very constant distribution, which in the cat varies only at the posterior border of the septum. The greatest variation in the area of any artery takes place in the distribution of the circumflex. The left margin, that adjoining the descendens, is uniform; its septal margin, however, may involve the whole posterior border of the interventricular septum and a portion of the right ventricular wall, or may be sharply limited by the posterior interventricular groove.

The margins of cardiac infarcts are not clear-cut and sharply defined, but quite irregular. They contain patches of apparently normal tissue, and are bordered by small infarcts scattered through the adjacent normal heart wall. This will be further discussed in the report of the histological details.

Comparison of the results of the physiological with those of the anatomical method. — It will be seen that the physiological distribution of the coronary arteries, obtained by the method of infarcts, corresponds in the main with the distribution outlined by anatomical methods. The physiological method is certain — the anatomical at best conjectural.

The physiological method defines accurately the relation of adjacent arteries, and the part which each plays in the nutrition of the portion of heart wall to which both are distributed. It demonstrates that two arteries may supply parts of the same area and still be independent of each other. Overlapping of this sort occurs particularly between the circumflex and the right coronary arteries in the posterior wall of the heart, and between both these vessels and the arteria septi in the posterior third of the septum.

The overlapping of the distribution of main stems and larger branches must be carefully distinguished from that of smaller branches. In the case of smaller branches, as has been shown by Kolster,[1] the area of infarction is not homogeneous, but consists of small foci of necrosis separated by masses of normal tissue. A method of distribution identical with this occurs at the margins of the larger infarcts, while the body of the infarct contains only completely necrotic material and no interspersed normal tissue.

IV. THE CONTRACTILITY OF THE ANÆMIC AREA.

Many observers have noted that the beat of the heart continues for a brief time after the closure of large branches of the coronary arteries, but no one has studied as yet the contractility during the first stages of infarction. The discovery that portions of the mammalian ventricle will resume their contractions if fed with defibrinated blood enables us to determine how long an ischæmic area in the heart remains contractile. A coronary artery must first be ligated, the animal kept alive until infarction has begun, and the ischæmic portion then cut out and fed with defibrinated blood through a branch of the coronary artery.

Experiment April 18, 1898. The descending branch of the left coronary artery in a dog was ligated in the manner already described. After an interval of six hours the dog was bled from the carotid artery, and the blood defibrinated, diluted with 0.8 per cent sodium chloride solution, and placed in a reservoir under pressure, in a warm bath heated to 37° C., according to the method described by Porter.[2] The heart was then extirpated. The area of distribution of the descendens was found to be pale grayish-brown, translucent, and sharply marked off from the contiguous normal tissue. A cannula was tied into the descendens below the ligature, and the anæmic area excised, care being taken not to include any of the normal tissue. The cannula was then connected with the reservoir of defibrinated blood and the piece of anæmic heart muscle perfused, under a pressure of 97 mm. Hg. The suspended muscle began to contract rhythmically within one minute after the blood stream had been established in it. Five minutes later it fibrillated, but after its blood-supply had been interrupted for several minutes, resumed its rhythmical contractions, and continued to beat regularly at 104 contractions per minute for one hour, when it was disconnected from its blood-supply.

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[1] KOLSTER: Skandinavisches Archiv für Physiologie, 1893, iv, p. 25.

[2] PORTER: Journal of experimental medicine, 1897, ii, p. 391, and This journal, 1898, i, p. 514. It is to be remarked that dilution with saline solution is a convenience — not a necessity. Undiluted blood will serve.

Experiment May 2, 1898. The same preliminary steps were taken as in the foregoing experiment. An interval of eleven hours was allowed to pass after the ligation of the artery. The dog was then bled as before and the heart removed. The anæmic area was opaque, distinctly yellowish in color, uniformly and sharply outlined. When, however, perfusion was begun, under a pressure of 60 mm. Hg., the excised anæmic muscle responded immediately with rhythmical contractions, weak at first, then growing stronger, and these continued uninterruptedly for thirty minutes. The contractions were markedly weaker in the centre of the area and increased in strength toward the periphery. The weaker contraction in the peripheral parts was evident also when the piece of muscle was cut into longitudinal strips, each strip including a branch of the nutrient artery. The strips from the peripheral portion of the area contracted more vigorously than the central ones. The precaution was taken in this experiment, as in the preceding one, to include in the preparation only the anæmic area.

From these experiments it will be seen that the part of the heart wall rendered anæmic by the interruption of its arterial blood-supply remains contractile at least eleven hours. How much longer contractility may persist has not been determined. The most recent infarct examined microscopically was twenty-two hours old, and its appearance indicated that contractility had been completely lost.

It has thus been demonstrated that (1) the heart muscle continues to contract for a time after the interruption of its circulation, and (2) it retains its ability to contract for a much longer period; whether it actually does contract for longer periods has not been determined. The contractions of the anæmic area may be accounted for in two ways: the energy for the contraction is either derived from some store of contractile material present in the muscle at the time the nutrient artery is ligated, or is supplied after ligation by the establishment of a feeble, temporary circulation, which becomes progressively less efficient and finally fails to prevent the death of the area. The sources from which this circulation may be derived are three; namely, the vessels of Thebesius, the coronary veins, and possibly to a slight extent the adjacent capillary systems. Of these the first two present conditions peculiar to the heart, and require discussion.

The coronary veins have a free anastomosis with each other, and, as Pratt[1] has shown, a direct communication with the vessels of Thebesius by vessels larger than capillaries. Both the coronary veins and the vessels of Thebesius are practically valveless. It may be assumed therefore that in them blood will flow in the direction of least resistance, whether with or opposite to the normal direction. In an inquiry into the influence of the heart-beat on the flow of blood through the walls of the heart Porter[2] has shown that each contraction compresses the intramural vessels. The force of this compression is very considerable. If the apical half of the ventricles of the dog's heart is removed and fed at a constant pressure with defibrinated blood through a cannula tied into the ramus descendens, blood from the severed arteries in the margins of the preparation will shoot far out with each contraction. This systolic compression of the intramural vessels is very important in our present problem. It is known that the anæmic area continues to contract for a time. The arterial supply being shut off, the systolic compression of the vessels within the wall in the anæmic area will empty them of blood. At the beginning of diastole these empty patulous vessels, in which the blood-pressure has been reduced to nothing or less than nothing, will be readily filled from the superficial coronary veins and the vessels of Thebesius. The former lie upon the surface — they are extramural — and are not emptied by the systolic squeeze; the latter open into all the cavities of the heart, and may draw blood directly from an always ready source. The quantities of blood which may be thus secured are relatively small but may have important effects. Magrath and Kennedy[3] have proved that the mammalian heart will beat on a surprisingly small supply, and reference has already been made to the fact that the mammalian heart can be kept beating by the blood drawn from either the vessels of Thebesius or the coronary veins. Finally, we are not without direct experimental evidence that the sources just discussed are of value. I find, as has already been mentioned, that the contractility of the anæmic area is least in the centre and greatest at the margins, which are more favorably placed than the central parts for receiving blood in the manner suggested. When a coronary artery is closed suddenly, these auxiliary sources never succeed in preventing coagulation necrosis, but it must be conceded that their value may be great in the many instances in which closure extends over months and years.

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[1] PRATT: This journal, 1898, i, p. 92.
[2] PORTER: *ibid.*, p. 145.
[3] MAGRATH and KENNEDY: Journal of experimental medicine, 1897, ii, p. 13.

V. GROSS AND MICROSCOPICAL CHANGES IN THE INFARCTS.

Kolster[1] has described the changes which take place when a small branch of a coronary artery of the heart is ligated. As a greater area and other relations are involved in the infarcts produced in the present investigation, it has been deemed necessary to study again the gross and microscopical changes in the area of infarction.

Histological method. — The cats operated upon were killed at periods of thirty hours, three, four, five, six, and fourteen days, two months, and seven months after the ligation; in dog hearts the intervals were twenty-two, thirty, and thirty-six hours, and ten, fifteen, and ninety days. A view of the progressive changes in the infarcts was thus obtained. The infarcted cat hearts were hardened *in toto* in Zenker's fluid forty-eight hours, washed for the same period in running water, and then rehardened in rising grades of alcohol, during which process they were passed through a seventy per cent solution of iodine. They were then imbedded in celloidin and each block was hardened first by moderate evaporation and then in eighty per cent alcohol. The blocks were mounted whole, and transverse sections varying from eighteen to thirty micromillimetres in thickness were cut from the apex toward the base. The sections were preserved in series, those of each millimetre being kept separately. Of these, one from each millimetre was stained and mounted, so that a series with intervals of one millimetre was obtained from each heart. Alum-hæmatoxylin and a one per cent aqueous solution of eosin were used as stains. Selected portions of infarcts in the dog's heart were treated in the same way, but no serial sections were made.

To determine the presence of fatty changes small pieces of infarct were hardened in Flemming's solution.

Gross changes. — Macroscopically the infarcted area becomes white or whitish yellow in color, opaque, and flaccid. On section it presents a smooth, bright surface. In three cases, one in the cat's heart and two in the dog's heart, the infarct was hæmorrhagic. In all cases the portion of the heart wall involved was dilated to a greater or less extent; the dilatation increases up to the fourth day. In all recent cases the veins arising in the infarcted area and their anastomosing branches remained intact; after the fifth day their presence was not noted in any case. Infarcts two weeks or more old became whiter, began to contract, and showed all the changes of newly developed connective tissue. In two cases polypous growths were present in the pericardium over the infarcted area. In the oldest case in the series (seven months) the infarct had been slightly reduced in size and had been converted into a thin, white, semi-transparent, glistening membrane, into which the muscular wall inserted as into a tendon. Neither in this case, nor in any other in the series, was the heart wall sufficiently dilated to form an aneurism. No clots or visible fibrinous sheets were found on the endocardium.

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[1] KOLSTER: Skandinavisches Archiv für Physiologie, 1893, iv, p. 1.

Histological changes in recent infarcts.—The microscopical examination sought to establish the character of the degeneration in the infarct, and the progressive changes which take place as the interval after ligation increases, and to determine whether degeneration was complete throughout the area or whether patches of tissue remained normal here and there.

Coagulation necrosis is found fully developed through the whole area of the infarct as early as twenty-two hours after ligation of the artery (dog, circumflex). At this hour many nuclei can no longer be stained, some show irregularities and fragmentation, and a few are swollen and vacuolated. In the earliest cases examined, the protoplasm had undergone granular degeneration, but in cases three or four days old hyaline degeneration is more frequent. Small vacuoles occur in many muscle cells, usually several in each cell so affected. In sections of pieces of infarct hardened in Flemming's solution, small black granules are found in some of the muscle cells. They are not present in many cells, but are very characteristic where they occur. The endocardial connective tissue, and the muscle cells bordering immediately upon it, are in some cases not involved in the general necrosis. The endocardium in these is normal; in other instances, however, it is covered with a thin membrane of fibrin, which encloses a few red corpuscles. At the margins of the infarct, and only there, groups of normal muscle fibres are scattered through the necrotic tissue, and sometimes acquire macroscopical size. These interruptions of necrotic area by normal tissue are identical with the infarcts which

Kolster produced by the ligation of a branch of the ramus descendens.

The condition of the principal blood vessels and the capillaries in the infarcted area varies greatly. Throughout the larger part the capillaries and small vessels contain no blood corpuscles and are difficult to distinguish. At the margins of the infarct they are usually distended with blood, and small extravasations of red blood corpuscles are sometimes present in the surrounding tissue. The vessels near the endocardium frequently share in the obliteration which has overtaken those in the greater part of the infarct. In some cases, however, some of these vessels are distended, and in one or two cases small hæmorrhages were observed in the centre of the infarct near the endocardium. The veins on the pericardial surface are always filled with blood.

In very recent cases the connective tissue in the centre of the infarct does not seem to be involved in the necrosis. Its nuclei retain their power of staining and are neither fragmented nor irregular. In older infarcts (three to five days) these have undergone degeneration.

Changes in the infarct with progressing age. — Infarcts three days old are little changed from the condition on the first day. Hyaline degeneration is more frequent, and cross striation less pronounced in the muscle fibres which retain their form than in more recent infarcts, and the connective tissue has in many cases become involved in the necrosis. The periphery of the infarct has become infiltrated with leucocytes, and the connective tissue cells both within the margin and just outside the infarct have begun to proliferate and extend slightly into it. The further changes up to about the sixth day consist in the steady penetration of leucocytes and newly formed connective tissue cells into the infarct, with absorption of portions of necrotic tissue. By the fourteenth day bands of connective tissue cells traverse the whole infarct, carrying capillaries with them. The mass of necrotic muscular tissue has almost entirely disappeared; connective tissue fibres have begun to be formed. Extravasated red blood corpuscles retain their outline even up to this time, and seem to be absorbed less easily than the necrotic muscular tissue. The newformed connective tissue is not confined to the limits of the infarct, but sends large strands into the normal muscular tissue, displacing some of it, and enclosing and isolating other portions.

It must be noted here that in almost all cases the larger vessels, both arteries and veins, are still intact, that is, their cells exhibit no alterations in the protoplasm or in the staining power of the nuclei.

The next stage examined was an infarct of two months, which was produced by the ligation of the ramus descendens. It did not give the results which might have been expected to follow the conditions noted in the earlier stages. Macroscopically, a thin, soft, narrow white band of scar tissue was found parallel to the anterior interventricular groove and a little to the left of it. Microscopically this consisted of a thin wall of muscular tissue, apparently normal, traversed from side to side by bands of connective tissue, which, however, nowhere formed the entire heart wall. In the septum the anterior third was replaced by a wedge-shaped mass composed of a firm, close-woven white areolar tissue, affording conclusive evidence that the ligature was successful. Whether the small extent of the anterior wall of the left ventricle which had become fibrous was due to anastomosis in that part or to regeneration of muscle remains a question. There is, however, no evidence for regeneration.

The histological changes in infarcts of the dog's heart are identical in character with those in the heart of the cat, and progress with equal rapidity. Local hæmorrhagic infiltration occurred more frequently in the dog than in the cat. In one case the infiltration took place in two narrow zones parallel to the endocardium. One of these lay just within the muscular layer, next the endocardial connective tissue; and the other, of about equal width, extended along the middle of the heart wall.

VI. CHANGES IN THE HEART'S ACTION FOLLOWING LIGATION.

The changes in the force and frequency of the heart-beat immediately following the ligation of a coronary artery were described by Porter in 1894.[1] In the present investigation, in a series of twenty-one cats operated upon, nine of which were not used in the foregoing portion of the investigation because they survived the operation only a few hours, the heart in thirteen gave no clear evidence of alteration in rate, in five showed a diminution in both force and frequency, and in three cases fibrillated; one of these recovered. In a series of nine dogs, the heart showed diminution in frequency of beat in five cases, but no change in force; there was no case of fibrillation. Accurate, extended observations of changes in force and frequency would have interfered with the main purpose of the investigation by making it more difficult to keep the animal alive.

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[1] PORTER: Journal of physiology, 1894, xv, p. 121.

At a later period after the operation the pulse became more frequent and weaker; the rhythm sometimes remained regular, but in many cases was irregular and often intermittent. This condition usually persisted for thirty-six to fifty-four hours, and then gave place to a pulse normal in rate and rhythm.

Auscultation of the heart sounds was performed only in dogs, as the heart of the cat beats too rapidly to make this profitable. The sounds remained clear and distinct, and no murmurs could be detected. The pitch was unchanged, and no undue valvular quality could be made out in the first sound.

Death from sudden cardiac failure in cases which survived more than twenty-four hours occurred twice, each time after violent exertion. The first case was in a cat whose circumflex artery had been ligated five days before, and happened in a quarrel with another cat; the other case occurred in a dog in which the descendens had been tied fifteen days before; his death followed soon after he had been fed and had been jumping about to be petted.

SUMMARY.

1. The coronary arteries are "terminal;" their anastomoses rarely permit the formation of a collateral circulation.

2. The physiological distribution of the coronary arteries obtained by the method of infarcts, corresponds in the main with the distribution outlined by anatomical methods.

3. The part of the heart wall rendered anæmic by the interruption of its arterial blood-supply may remain contractile at least eleven hours after the ligation of the artery.

4. The central part of the anæmic area undergoes coagulation necrosis more rapidly than the peripheral parts. Probably the outlying portions receive blood through the vessels of Thebesius and the coronary veins.

5. In animals which survived the ligation of a large coronary trunk thirty-six to fifty-four hours the pulse seemed normal in rate and rhythm and the heart-sounds were clear and distinct. No murmurs could be detected and no undue valvular quality could be made out in the first sound.

6. The results of the study of the anatomical distribution of the coronary arteries, the investigation of their anastomosis with the Röntgen rays, their physiological distribution determined by the boundaries of the infarction following their closure, and the gross and microscopical changes in the infarcts, cannot be stated in a brief summary, but must be sought in the text.

In conclusion I wish to express my indebtedness to Prof. W. T. Porter, at whose suggestion and under whose direction this investigation was carried on.

ON THE CAUSES OF THE ORDERLY PROGRESS OF THE PERISTALTIC MOVEMENTS IN THE ŒSOPHAGUS.

BY S. J. MELTZER.

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IT is now generally assumed that the orderly progress of the peristalsis in the œsophagus is exclusively of central origin. This means that the first afferent impulse which is conveyed from the periphery to the centre of deglutition and which causes the co-ordinate contraction of the mylohyoid, pharyngeal, and laryngeal groups of muscles, travels further within the centre through several groups of ganglia, sends down successively along its route efferent impulses to the several divisions of the œsophagus, including the cardia, and causes hereby their successive contractions without the aid of new afferent stimuli. This view is based upon observations which demonstrate that the lower part of the œsophagus as well as the cardia continues to show the peristaltic contractions even after the ligation or division of the œsophagus. The observations upon the cardia were made by Kronecker and myself[1] and stand uncontradicted. The observations upon the œsophagus were made by A. Mosso,[2] and are diametrically opposed by the earlier experiments of Wild,[3] done in 1847 under the direction and special participation of Ludwig. Wild states in unmistakable terms that strong compression, ligation, or division of the œsophagus positively stops the progress of the peristalsis, and from his experiments he draws a conclusion just the opposite of ours, namely, that the peristalsis is caused by reflexes from the periphery, that is, from each part of the œsophagus itself. Mosso, who was stimulated to his researches on the peristalsis of the œsophagus by these very statements, reports simply his own experiments without offering any explanation of the strange contradiction between himself and Wild. So far as I know, this subject has since Mossó not yet been reinvestigated, and although we ourselves have supported the view of Mosso, as harmonizing with our own, our experiments themselves were restricted to the cardia. A fresh study of this subject, which I have recently taken up again, makes it probable that the contradiction between Mosso and Wild exists rather in the conclusions drawn from the facts than between the facts themselves, inasmuch as the purpose and the method of these investigators were not exactly the same. Wild experimented upon the cervical œsophagus while Mosso studied the movements in the thoracic part; again, Wild studied the movements by direct observation, while Mosso judged whether peristalsis were present by the motion of a wire attached to an olive-shaped body which was introduced in the upper part of the thoracic œsophagus; finally, Mosso used ether for anæsthesia, while Wild injected opium in the jugular vein. With these points in my mind I started a series of experiments regarding the nature and the causes of the peristalsis in the œsophagus which have brought noteworthy results, some of which I shall now report briefly, illustrating them by the account of a few experiments.

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[1] KRONECKER and MELTZER: Archiv für Physiologie, 1883, Suppl. Bd., p. 328.

[2] MOSSO, A.: Untersuchungen zur Naturlehre, 1876, xi, p. 327.

[3] WILD: Zeitschrift für rationelle Medizin, 1847, p. 76.

I have in the first place repeated the experiments of Wild with the unimportant deviation that I used for anæsthesia morphine instead of opium. Here is an illustration.

Under local anæsthesia by cocaine the external jugular vein of a dog weighing nine kilos was exposed and 0.06 gram of morphine injected. This produced a fair anæsthesia, which lasted throughout the experiment. A cannula was inserted in the trachea and then the cervical œsophagus from pharynx to sternum exposed to full view. After each of the spontaneous deglutitions which occurred from time to time a well-defined peristaltic wave was seen passing down the entire visible length of the œsophagus. Mechanical stimulation by pressing the mylohyoid region or the larynx or by tickling the soft palate and pharynx with a rod introduced through the mouth failed to bring out any deglutition. However, by pouring water into the mouth assisted by pressure upon the mylohyoid region the animal could be made to swallow two or three times, the deglutitions usually following each other in rapid succession, and here the peristaltic wave was seen to sweep down the œsophagus only after the last swallow. A tight ligature was now put around the œsophagus at a distance of about six centimetres from the larynx, and the animal was made to swallow. The portion of the œsophagus above the ligature became distended immediately; there was no peristalsis below the ligature following this swallow. The animal, however, soon started without any new stimulus to swallow incessantly, and the upper portion of the œsophagus began to swell to large proportions. Apparently there was a great effort, in which even the muscles of the neck seemed to take part, to force the swallowed mass through the ligature. The rapid swallowing and the entire forcing effort could be checked for a while by holding down the larynx, and from time to time there was also a spontaneous pause for a minute or two; but at no time could a peristaltic movement be discovered in the portion of the œsophagus below the ligature. As soon, however, as the ligature was opened the mass came down immediately the whole visible length of the œsophagus with a distinct peristaltic wave, without the occurrence of a new swallow. When the animal was now made to swallow by pouring water into the mouth, a peristaltic wave was again seen to run down the entire œsophagus after the last swallow. The ligating of the œsophagus and the opening of the ligature were repeated a number of times with invariably the same results. It is hardly necessary to state expressly that care was taken not to include the recurrent nerve in the ligature.

This experiment, then, brings out a simple confirmation of the statements of Wild with regard to the peristaltic movement in the cervical œsophagus.

In the following experiment in addition to the direct observation of the behavior of the cervical œsophagus, the method of Mosso was also employed to study simultaneously the action of the thoracic part of the œsophagus.

A dog of eight kilos was anæsthetized in the manner described in the previous experiment, the cervical œsophagus freely exposed, and a strong thread put loosely around it at a distance of six centimetres from the larynx. Below the thread a longitudinal incision was made in the œsophagus and an olive-shaped body of hard rubber introduced through it into the thoracic portion of the œsophagus. To this body was attached a long silk thread which passed over an elevated projection and was slightly stretched by a light weight, affording thus a means by which the movement of the olive-body could be easily observed. The introduction of the body caused no peristalsis of the thoracic œsophagus — the thread did not move. A spontaneous deglutition or a deglutition caused by water in the mouth was followed by a peristaltic wave in the œsophagus, but only to the longitudinal incision. Here some liquid escaped and no peristalsis was seen in the part of the cervical œsophagus below the incision nor did the thread move. The longitudinal incision was now closed by a few stitches, but so as still to permit the free movement of the silk thread attached to the olive-body. When now a peristaltic wave started in the œsophagus it promptly went down to the sternum, and soon the olive-body could also be noticed moving downward to the stomach. In this animal mechanical stimulation by a rod introduced into the mouth often caused deglutition, but the latter was mostly restricted to the contractions of the mylohyoid, pharynx, and larynx, and rarely followed up by peristalsis in the œsophagus. By pouring water into the mouth, however, the characteristic ascent of the larynx was promptly followed by a distinct peristaltic wave in the œsophagus. The thread around the œsophagus was now tightened and the animal was made to swallow once. Immediately the entire complex of incessant swallowing, extreme distention of the part of the œsophagus above the ligature, and considerable efforts to force the passage, appeared on the scene in the manner described in the previous experiment; but here, too, at no time was there any peristaltic movement visible in the part of the cervical œsophagus below the ligature, nor was there any movement of the thread attached to the olive-shaped body. After loosening the ligature a wave immediately swept down the œsophagus, and the thread also indicated a downward movement of the olive-body. The tying and untying of the thread around the œsophagus was repeated a number of times with the same result. Finally a longitudinal incision was made in the upper part of the œsophagus. Now the peristalsis came down only to the incision, while the rest of the cervical œsophagus as well as the olive-body never moved. When now the œsophagus was ligated the swallowing did not produce any distention of the œsophagus, liquid and air escaped through the incision, and no incessant swallowing took place.

This experiment shows that when the œsophagus is ligated there is apparently neither in the cervical nor in the thoracic part a peristaltic movement, which would seem to be a direct contradiction of the statements of Mosso. In both these experiments, however, the animal was narcotized by morphine. To make the conditions of our experiments uniform with those in the experiments of Mosso we have conducted some of the experiments under ether anæsthesia. Following is an example.

A dog of about seven kilos was anæsthetized by ether in the usual way, then tracheotomy was performed, and ether given by tracheal cannula. The œsophagus was exposed and the olive-body introduced as in the previous experiment. The animal was now under such deep anæsthesia that water poured into the mouth remained there, and no stimulus would cause any deglutition. Anæsthesia was diminished, and the animal was allowed to come out of its influence gradually. The first effective stimuli brought out deglutitions which were not followed by any peristalsis in the œsophagus; next came a stage when deglutition caused by pouring water into the mouth was followed by peristalsis, but no peristalsis was seen after "empty" swallows brought out by mere mechanical stimulation. Finally a stage came in which the peristalsis took place promptly after every deglutition caused by any kind of stimulus.

The dog was now in a state of light anæsthesia. The œsophagus was now ligated and the lively swallowing and the swelling up of the upper part of the œsophagus appeared as in the previous experiments, but no peristalsis occurred below the ligature, nor did the silk thread indicate any motion of the olive-body. The experiments with ligation were repeated several times with the same results. Then the œsophagus was cut transversely at the middle of the neck; the lower end retracted into the thorax. When now the animal was made to swallow by pouring water into the mouth or by any other method, the silk thread regularly indicated the descent of the olive-body into the stomach about three to four seconds after each swallow, or after the last one, when deglutitions followed each other in rapid succession.

Here then is an experiment which also confirms the statements of Mosso. It should be added that in nearly all his experiments, Mosso tried to interrupt the progress of the peristalsis mainly by division of the œsophagus or by removing a part of it; the ligation he tried only in a single instance, but then he applied a method quite different from that which Wild and I have employed. I have reason to believe that this difference in the method may be of some importance to our question, but I am not prepared to discuss this point at present.

These few experiments teach us that the statements of Wild as well as of Mosso are both correct (and, what is more, even both conclusions seem to be correct), giving us at the same time the key of the apparent contradiction. We have seen that the appearance of the peristalsis in the œsophagus is considerably influenced by the degree of anæsthesia to which the animal is subjected.

At a certain deep degree of anæsthesia no peristalsis follows the first act of deglutition.

Then comes a stage when the peristalsis appears in the œsophagus only after such deglutitions as are caused by the presence of water in the mouth. If the deglutition be caused by a mechanical stimulus with very little or with no liquid thrown into the œsophagus, no peristalsis takes place. If the water thrown into the œsophagus can escape through a longitudinal or transverse cut or finds an obstacle in a ligation, the peristalsis following the swallowing of water progresses just to the cut or the ligature, and no further. This degree of anæsthesia apparently affects the centre of deglutition in such a manner as to stop the progress of the primary afferent impulse within the centre, or to prevent the transmission of its efferent impulses to the œsophagus. The appearance and the progress of the peristalsis can now be maintained by direct, short-circuit, reflex contractions caused by the swallowed mass while travelling along its route to the œsophagus.

With a moderate degree of anæsthesia the centre of deglutition is not materially constrained in its function, and the successive contractions of the different parts of the œsophagus are attended to by the primary afferent impulse which started the entire act of deglutition; therefore even an "empty" deglutition is followed by a peristaltic wave, and therefore the peristalsis continues its course even into the part below the division of the œsophagus.

Wild has employed morphine and Mosso ether — not that there is necessarily such a fundamental difference between these two drugs in their anæsthetizing power; the important difference in our case is, as I believe, merely an accidental one. Morphine leaves the body quite slowly, while the animal gets out of the ether-anæsthesia pretty quickly, especially when it can breathe without the obstacle which the glottis and the obstructing parts above it offer to the expiration, *i. e.*, when it breathes through a tracheal cannula. An animal can be deeply anæsthetized through a tracheal cannula within a few minutes and can be out of the anæsthesia in an equally short time. Mosso in nearly all his experiments had the trachea divided, and in some experiments he expressly points out that the animals were almost entirely out of their anæsthesia. Wild on the other hand expressly states that his aim was to have the animal under good anæsthesia, since animals are then, he affirms, reliable reflex-preparations. Wild, in short, observed in his experiments conditions which prevail during deep anæsthesia, and thus discovered the reflex action originating within the œsophagus itself, but failed to see the behavior of the centre of deglutition in normal or nearly normal conditions. Mosso, on the other hand, working under nearly normal conditions, discovered the central nature of the normal progress of the peristalsis, but did not become aware of the existence also of direct reflex mechanisms in the œsophagus, which are perhaps of some assistance even in normal conditions, and surely are of importance as reserve forces when the centre of deglutition is for some reason constrained from exercising its superior function.

I have to add one remark. There might seem to exist some discrepancy regarding a certain point between my statements and those of Wild. I have often seen quoted Wild's assertion that a longitudinal incision does not prevent the progress of the peristalsis, while in my experiments I have often observed that where a transverse cut prevents the progress of the peristalsis there also a longitudinal incision will prevent it. Wild, however, has made the longitudinal incisions only through the muscular sheaths, taking special care not to injure the mucous membrane, while the incisions I am speaking of were made through the mucous membrane also, thus purposely facilitating the thorough escape of the swallowed masses.

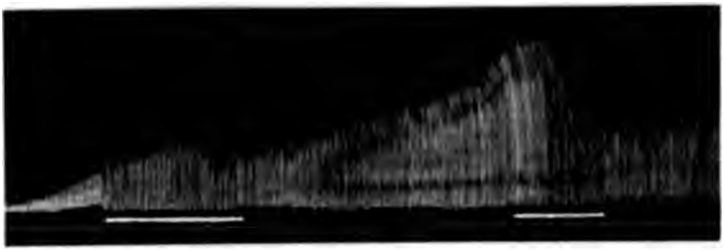

Fig. 2. Supra-renal Extract. (From right to left.

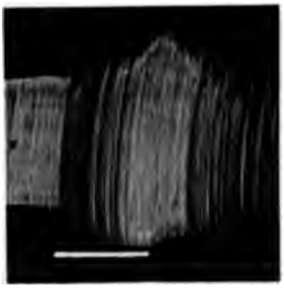

Fig. 3. Kidney. (From left to right.)

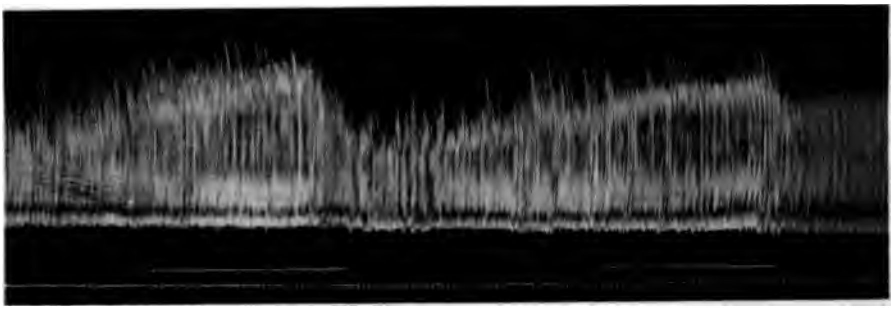

Fig. 4. Thyroid. (From right to left.)

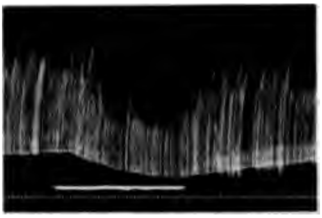

Fig. 5 Thyroid. (From left to right.)

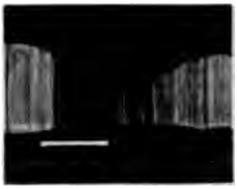

Fig. 6. Anthrax Culture. (From left to right)

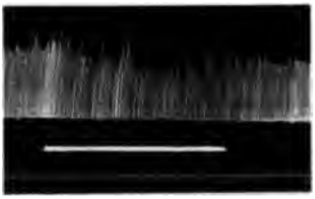

Fig. 7. Filtrate of S. Pyog. Aureus. (From left to right.)

Curves drawn by the "apex" of the dog's heart; one-half the original size. Time in *five-second* intervals. The white line gives the duration of perfusion.

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THE ACTION OF ANIMAL EXTRACTS, BACTERIAL CULTURES, AND CULTURE FILTRATES ON THE MAMMALIAN HEART MUSCLE.

BY ALLEN CLEGHORN.

[From the Laboratory of Physiology in the Harvard Medical School.]

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THE present research on the isolated apex of the dog's heart was undertaken in order to determine the immediate action of glandular extracts and the cultures and filtrates of bacteria on the heart muscle itself.

The influence of nerve cells has been eliminated by using that portion of the heart from which they are absent. The freedom of the apical half of the ventricles from ganglion cells has been recently demonstrated once more by the careful research of Schwartz,[1] in which serial sections of the ventricles of the heart were made in three different planes and new methods of staining employed; nerve cells were found in the ventricles only in the basal third. Any changes produced in the apex preparation by the action of the different substances perfused through it must then of necessity be due to changes in the cardiac muscle itself. There is at present no reason to suppose that these agents act on the heart muscle by stimulating its nerve fibres.

METHOD.

The action of the various animal extracts, filtrates, and bacterial cultures upon the heart muscle free of nerve cells was studied by recording the contractions of the apical portion of the ventricle of the dog's heart kept beating by feeding it with blood according to

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[1] SCHWARTZ: Archiv für mikroskopische Anatomie, 1898, liii, p. 63.

Porter's method[1] before, during, and after the perfusion of the apex with the substance to be tested mixed with defibrinated blood.

The preparation of the animal. — The dog received 0.03 to 0.06 gram morphine sulphate hypodermically and fifteen minutes later was anæsthetized with ether. Blood was drawn from the left carotid artery until dyspnœa appeared, when the bleeding was stopped, and a quantity of normally warm 0.8 per cent sodium chloride solution equal to the blood withdrawn was allowed to flow into the right jugular vein. After the saline solution had circulated a few minutes the animal was bled again until dyspnœic. The thorax was hastily opened and the still beating heart rapidly removed and placed in a beaker of warmed normal saline solution, in which it usually continued to contract for some minutes. The blood from the first bleeding was thoroughly defibrinated, filtered twice through glass wool, and divided into portions, one of which was mixed with the product of the second bleeding, while to the other was added an equal quantity of a sodium chloride solution (0.8 per cent) containing the desired proportion of the substance to be tested.

In more than half the experiments, however, the dogs were so large that it was unnecessary to perfuse them in the manner described; it sufficed to bleed them once and to dilute the blood thus gained with an equal quantity of saline solution. A small portion of the undiluted blood was reserved for mixture with the extract of the substance to be tested, which again was always added in such a way that the percentage of blood should be the same in the " normal perfusion fluid" and in the perfusion fluid containing the extract.

A very small glass cannula was now tied into one of the small branches of the coronary artery going to the apex, usually the descendens, and the portion of the ventricle supplied by the branch was cut out and placed in a warm chamber. The cannula was joined to a perfusion tube, and the lower end of the strip of ventricle connected with a writing lever. In some large animals in which the distribution of the arteries was especially favorable as many as three apical preparations could be made from the same heart.

The perfusion apparatus. — The apparatus for carrying on perfusion of the apex, shown in Fig. 1, consisted of a water bottle, a pressure bottle, two blood reservoirs, — one for normal blood and one for blood containing the agent to be tested, — and a chamber in which the apex could be maintained at a constant temperature. The blood reservoirs and the chamber for the apex, with the tubes connecting them, were surrounded with water in a galvanized iron tank. In the upper right hand corner of Fig. 1 is seen the water bottle, supplied with water from a tap, and slung from the ceiling so as to be raised or lowered at will. In the tubulure was fixed a brass tube connected by thick-walled rubber tubing with the pressure bottle. The brass tube was provided with a vertical limb 15 cm. high, open to the air to prevent siphonage. Five centimetres from the top of the vertical limb was a side branch through which the excess of water escaped into a sink. The water in the bottle was thus maintained at a constant level. The pressure bottle is seen on a stand a little to the right of the centre of the figure. Through the cork passed two tubes; one conveyed water from the water bottle already described, the other conveyed the compressed air to the blood reservoirs. The larger blood reservoir contained the normal perfusion fluid, while the smaller held the substance to be tested mixed with defibrinated blood in the desired proportion. Through the stopper of this small reser-

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[1] PORTER: Journal of experimental medicine, 1897, ii, p. 391.

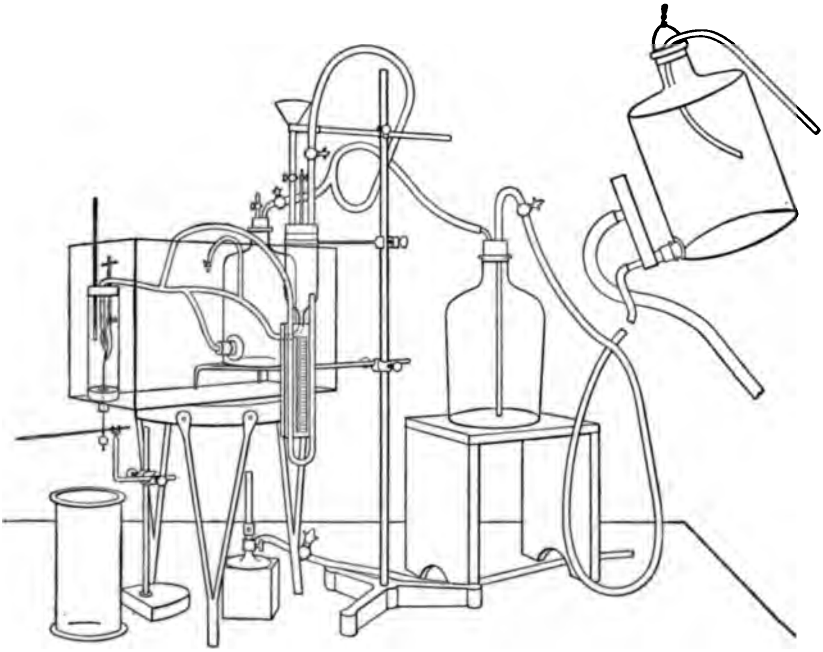

FIGURE 1. Perfusion Apparatus.

voir ran a glass funnel, which allowed the bottle to be filled without the removal of the stopper. The perfusion tubes given off by the reservoirs united to form the main perfusion tube. The tube from the small reservoir, before it joined with that of the normal reservoir, gave off a side branch, the end of which was closed by a small stopcock; this allowed the small bottle to be washed out without interrupting the normal perfusion, so that each toxic substance could be thoroughly removed before another was added. About five centimetres beyond the junction of these perfusion tubes the main tube gave off a branch to a mercury manometer by which the pressure of the perfusion fluid was recorded. The main tube then ran directly to the chamber for the apex — seen at the left of the tank with a piece of muscle ready for perfusion — where it was connected with the cannula in the branch of the coronary artery. In the stopper of the warm chamber were fixed a thermometer and an adjustable clamp to hold the strip of apex in position. The lower end of the apex chamber had a small opening for the escape of the blood which had passed through the vessels of the preparation. This opening served also for the wire connecting the apex with the recording lever, which was of the third class and magnified seven times. When the apparatus was in use the heart chamber, blood reservoirs, and perfusion tubes were submerged in water of a temperature of 37.5° C. No reference has been made to the stopcocks seen in Fig. 1, as their uses can readily be understood. No stopcocks were used on the perfusion tubes, the circulation through them being controlled with long-handled artery forceps. The recording lever marked on the smoked surface of a Baltzar drum revolving very slowly. An electrical time marker was used to indicate every five seconds.

The preparation of the glandular extracts. — The glandular extracts were in all cases prepared from perfectly fresh material dissected from newly killed sheep at the slaughter house.

Both glycerine and saline extracts were employed. The former is invariably the more powerful.

In preparing the glycerine extract the fresh glands were stripped of fat and other extrinsic material, weighed while in a moist state, cut into small pieces, placed in a glass vessel, and covered with chemically pure glycerine in the proportion of one gram of gland substance to one cubic centimetre of glycerine. After allowing the gland to remain in this for twenty-four hours the glycerine extract was strained through cheesecloth and added to boiled 0.8 per cent sodium chloride solution in the desired proportion. The mixture was then filtered, and on the addition of an equal quantity of pure defibrinated blood was ready for perfusion. The perfusion fluid always consisted of fifty per cent blood and fifty per cent normal saline solution, but the quantity of glycerine extract dissolved in the saline solution varied. The usual proportion was glycerine extract 5 c.c., saline solution 45 c.c., blood 50 c.c.

The saline extracts were prepared by rubbing up the desired quantity of the gland substance in a mortar with 0.8 per cent sodium chloride solution and a small quantity of clean white sand. The mixture was then gently warmed, filtered, and added to the blood in the proportion required. No extracts were prepared from dried glands.

The preparation of cultures and filtrates. — The cultures of bacteria used in these experiments were grown on dog's serum. An attempt to use bouillon cultures had to be abandoned because the presence of small traces of the calcium and potassium salts in the fluid so altered the contractions as to make an accurate judgment of the effects of the bacteria or filtrates impossible.

The serum for the cultures was secured by bleeding the animal into a sterilized vessel, which was set aside, protected from infection by having its mouth covered with several sheets of sterilized paper, until the serum had separated. The serum was then drawn off and put in several sterilized flasks, which were placed in an incubator and kept at a constant temperature (38° C.) for seven days. If at the end of that time the serum remained sterile the bacteria were planted in it and the cultures allowed to grow in an incubator for ten days. Then the contents of each flask was divided into two portions; one was left as it was, the other was passed through a Chamberland filter to remove the bacteria. This was done to determine if any difference in the character or strength of action existed between a culture containing the bacteria and its filtrate. Both the culture and the filtrate were added respectively to the normal saline solution, as already described in the case of the glandular extracts.

Control experiments were done with all the glycerine, saline solutions, and serum employed in the research. The first two substances gave no results; the serum usually increased very slightly the force of the contractions.

The perfusion fluid used in these experiments was the animal's own defibrinated blood mixed with an equal quantity of sodium chloride solution (0.8 per cent). The apex contracts equally well when perfused with its own undiluted blood, but the addition of the saline solution secures certain advantages; it gives a larger quantity of fluid to work with, an important matter when the experiment occupies six or seven hours; it also allows the experimental substance to be added to the perfusion fluid at the expense of the inert saline solution, and so does not alter the percentage of defibrinated blood. In control experiments it was found that the saline solution had no action on the contracting apex of itself, and when mixed with the defibrinated blood in varying proportions its only action was that of a diluent. That is to say, pure defibrinated blood gave strong apical contractions and the contractions were not affected in any appreciable degree when saline solution was added to the extent of fifty per cent; but beyond that the contractions of the apex became correspondingly weaker as the percentage of saline was increased, and on the perfusion of pure saline no response or at best a very weak and short-lived activity was developed.

In all experiments the blood was examined under the microscope to see if the experimental substance had produced any change in the corpuscles. In only one case was any noteworthy change detected; in this instance a culture of glanders had been added to the blood, and the corpuscles showed a tendency to stick together and so form small clumps. The extracts of glands did not alter the usual characters of the corpuscles; in all cases they looked entirely normal.

In these experiments the apex was kept at a constant temperature, namely, that of the body, and the pressure of the perfusion fluid was also constant — 80 mm. Hg.

With regard to the behavior of the apex preparation under normal perfusion (defibrinated blood diluted with an equal part of normal saline solution) it was found, in the greater number of cases, that the contractions were fairly regular in rate; the force varied more than the frequency, but not to such an extent as to impair the accuracy of the conclusions drawn in this paper. In one experiment only was the apex perfused with an animal extract while the contractions were irregular, and this was done merely to show the regulating influence of the extract (thyroid) on the rhythm.

An excess of the substance perfused sometimes caused the apex to fibrillate, but by entirely shutting off the perfusion and so asphyxiating the apex for a short time it was found that when the normal perfusion was resumed the apex as a rule returned to its regular contractions. This method of recovering the apex from fibrillary contractions was first pointed out by Langendorff.[1]

During the course of this inquiry thirty dogs were used; always two and sometimes three preparations were made from each heart. The extract of each of the glands investigated was used upon a fresh apex preparation, — one not previously employed for any purpose, — but more than one perfusion was made with each extract. Sometimes perfusion was done also on an apex which had been perfused before with some other extract, but this only when the apex had not been visibly affected or had recovered from the action of the foregoing substance. The table on page 280 gives the number of perfusions done with each substance.

THE ACTION OF THE EXTRACTS OF VARIOUS ORGANS ON THE HEART.

Suprarenal bodies. — The action of suprarenal extracts on the circulation was first fully demonstrated by Oliver and Schäfer,[2] who pointed out that its influence on the heart and blood pressure is largely a direct one, and that the medulla of the gland contains the active substance which on injection causes a rise in blood pressure and augmentation of the heart-beats. Among the more important subsequent investigations may be cited Hedbom's[3] research with the whole isolated cat's heart, in which the extract was perfused through the coronary vessels; here it was found that the suprarenals exerted a strong tonic influence on the contracting heart, and that the effect lasted for a long time. Cybulski,[4] Szymonowicz,[5] and Gottlieb[6] state that the extract of these bodies affects the heart through the nervous system. The last named observer pointed out that the substance is also a powerful cardiac stimulant; he revived the heart of a rabbit with suprarenal extract from the arrest brought on by the intravenous injection of chloral hydrate.[7] Vincent[8] has demonstrated on dogs that the suprarenals have no power when fed to animals even in enor-

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[1] LANGENDORFF: Archiv f. d. ges. Physiol., 1895, lxi, p. 319.

[2] OLIVER and SCHÄFER: Journal of physiology, 1895, xviii, p. 230.

[3] HEDBOM: Skandinavisches Archiv für Physiologie, 1898, viii, p. 147.

[4] CYBULSKI: Akademie d. Wissensch., Cracovie, 1895, 4 Mars.

[5] SZYMONOWICZ: Archiv f. d. ges. Physiol., 1896, lxiv, p. 97.

[6] GOTTLIEB: Archiv f. exper. Pathol. und Pharmakol., 1896, xxxviii, p. 99.

[7] GOTTLIEB: *Ibid.*, p. 106.

[8] VINCENT: Journal of physiology, 1898, xxii, pp. iii, 270, lvii.

SUBSTANCE.	TOTAL NUMBER OF PERFUSIONS.	ON FRESH APEX PREPARATION.		ON APEX ALREADY USED.	
		First Time.	Repetitions.	Times.	Previous perfusion.
Extract.					
Suprarenal	11	7	3	1	Thyroid extract.
Pituitary body . . .	6	2	2	2	Testicular extract, infundibular extract.
Infundibular body .	8	2	2	4	Testicular extract (4).
Testicle	11	5	2	4	Pituitary, infundibular, and thyroid (2) extracts.
Liver	9	6	1	2	Pancreas, spleen.
Pancreas	8	6	1	1	Submaxillary gland.
Submaxillary gland .	9	6	2	1	Spleen.
Spleen	8	6	1	1	Liver.
Kidney	8	4	4	0	
Thyroid	24	10	10	4	Testicular extract (2), suprarenal extract (2).
Culture or its Filtrate.					
Staphy. pyo. aureus .	6	3	3	0	
Culture filtrate . .	4	2	2	0	
Typhoid	5	1	1	3	Glanders, prodigiosus, aureus.
Culture filtrate . .	4	1	1	2	Capsulatus, aureus.
Prodigiosus	4	1	1	2	Aureus (2).
Culture filtrate . .	1	0	0	1	Prodigiosus.
Glanders	2	1	0	1	Typhoid.
Culture filtrate . .	3	1	0	2	Glanders, capsulatus.
Diphtheria	2	1	1	0	
Diphtheria toxine .	5	3	2	0	
Cholera spirillum . .	3	1	1	1	Aureus culture filtrate.
Culture filtrate . .	1	0	0	1	Aureus.
Capsulatus	5	1	2	2	Anthrax, tetanus toxine.
Culture filtrate . .	2	1	1	0	
Anthrax	5	2	1	2	Tetanus toxine, pyocyaneus.
Culture filtrate . .	3	2	0	1	Diphtheria toxine.
Pyocyaneus	2	0	0	2	Cholera spirillum, aureus.
Culture filtrate . .	1	0	0	1	Aureus culture filtrate.
Megatherium . . .	2	0	0	2	Coli communis (2).
Culture filtrate . .	1	0	0	1	Diphtheria toxine.
Coli communis . .	2	0	0	2	Prodigiosus, anthrax.
Culture filtrate . .	1	0	0	1	Diphtheria toxine.
Ramosus	2	0	0	2	Anthrax culture filtrate.
Culture filtrate . .	0	0	0	0	
Tetanus toxine . .	5	2	1	3	Pyocyaneus culture filtrate, anthrax culture filtrate.

mous quantities, but the giving of a minute dose intravenously immediately causes marked changes in the cardiac action and in the blood pressure.

The nature of the active principle of these bodies has been much discussed. Mühlmann[1] attributed the action to the presence of pyrocatechin. Langlois[2] contradicts this, and in an interesting paper in collaboration with Charrin[3] shows that the suprarenal extract is capable of diminishing the poisonous effects of nicotine. Abelous[4] points out that it is also antagonistic to the action of atropine.[5]

Of all the glandular extracts used in my experiments that of the suprarenal capsules is by far the most powerful; in the seven experiments done with this substance not once did it fail to produce a marked augmentation of the contractions of the apex.

In the experiment illustrated by Fig. 2, Plate I, the dog was anæsthetized with morphia and ether at 9 A. M. Fifteen minutes later he was bled from the left carotid artery. At 9.45 the heart was removed and at 9.52 normal perfusion was commenced; the apex showed strong contractions as soon as the perfusion began. At 9.59 the suprarenal mixture was allowed to pass through the apex. This liquid consisted of 3 c.c. of a glycerine extract made from the mixed gland, *i. e.* cortex and medulla, 47 c.c. sodium chloride solution (0.8 per cent), and 50 c.c. defibrinated blood. At 10.01 this toxic fluid was shut off and normal perfusion resumed; after six minutes the suprarenal extract was again turned on for three minutes, but, as the apex showed great excitement, resort was had to the normal perfusion fluid, — too late, however, to prevent the apex from fibrillating, as can be seen at the end of the tracing.

In all experiments the apex answered almost immediately to the presence of the extract, the contractions being often more than double the height of the normal ones (see Fig. 2) while the rhythm was slightly quickened. No alteration in the tonus was noticed unless the perfusion was carried on for some time, when a gradual rise took place until the apex ran into fibrillary contractions. Provided the first dose was of short duration, about two minutes, and not too strong, the effect gradually wore off, and, as is shown in Fig. 2, the heart did not respond to a second dose in so marked a manner or so readily as to the first. A strong dose of the extract, about ten per cent (saline extract), would as a rule cause the apex to fibrillate after giving four or five enormous contractions.

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[1] MÜHLMANN: Deutsche medicinische Wochenschrift, 1896, xxvi, pp. 409–411.

[2] LANGLOIS: Archives de physiologie, 1897, xxix, p. 152.

[3] CHARRIN and LANGLOIS: Bulletin de la société de biologie, 1893, p. 842, 1894, p. 99, 1896, p. 131.

[4] ABELOUS: C. r. de la société de biologie, 1896, p. 458.

[5] Since writing the above I have had the pleasure of hearing Dr. J. J. Abel's paper, read before the American Physiological Society, December 28, 1898, on the active principle of the medulla of the suprarenal glands. This epinephrin, as Dr. Abel calls it, has the formula $C_{17}H_{15}NO_4$ and is to be classed with the alkaloids. Its sulphate is very active; a dose of 0.00013 gram, equal to 0.00009 gram of the free base, produced a rise of 14 mm. Hg in the blood pressure in a small dog. Dr. Abel's paper will be found in the Bulletin of the Johns Hopkins Hospital, and in full in the Zeitschrift für physiologische Chemie, 1899.

Hypophysis cerebri. — Oliver and Schäfer[1] called attention to the action of the extract of the hypophysis when injected into the circulation. They obtained marked results, the blood pressure rising considerably, the heart's action being slowed, and the contractions augmented; a large dose was required to produce these effects, however. Mairet and Bosc[2] failed to obtain any pronounced effects after injections of the hypophysis. Hedbom[3] found in his research a tonic influence on the heart and a decrease in the frequency of the contractions. Cyon[4] found a slowing action on the heart in consequence both of stimulation of the hypophysis *in situ* and of injections of its extract. He believes that the active substance, though in the main similar to that of the thyroid, differs in that it contains phosphorus.

Under the heading " Hypophysis cerebri " I have thus far included two different structures, namely, the pituitary body proper and the infundibulum. The necessity of considering these structures separately has been pointed out by Howell,[5] who calls attention to the fact that all experiments with the hypophysis, previous to his own, have evidently been done with the infundibular and pituitary bodies combined.

In separate experiments with extracts of these two structures Howell found a great difference in their action. Little or no result was obtained with the pituitary body, while an injection prepared from the infundibular body gave constant and well marked effects, slowing the rate of contraction of the heart and raising the blood pressure. Moreover, it has been shown by Haller[1] and Berkley[2] that the histological structure of the infundibulum and pituitary body differs widely and that their embryological origin is dissimilar also. Berkley found in the infundibular body not only nerve structures but also closed vesicles lined with gland-like epithelial cells and containing a material like colloid. This substance has been shown by Howell to possess physiological activity.

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[1] OLIVER and SCHÄFER: Journal of physiology, 1895, xviii, p. 277; also SCHÄFER: British medical journal, 1895, Aug. 10; and Text-Book of physiology, edited by Schäfer, 1898, i, p. 946.

[2] MAIRET and BOSC: Archives de physiologie, 1896, viii, p. 600.

[3] HEDBOM: Skandinavisches Archiv für Physiologie, 1898, viii, p. 147.

[4] CYON: Archiv f. d. ges. Physiol., 1898, lxx, p. 126.

[5] HOWELL: Journal of experimental medicine, 1898, iii, p. 245.

These experiments confirm Howell's observations on the difference of action between these two substances. The perfusion of a glycerine extract (10 per cent) of the pituitary body alone caused an almost immediate change in the contractions of the apex; the frequency was considerably lessened but the amplitude of the contractions was increased; no alteration in tonus was noticed, but a small alteration may have been masked by the slight irregularity in the action of the apical preparation. No after effects were observed, the ventricular strip returning to its normal action on resuming normal perfusion. The change in the amplitude and frequency of the contraction began to disappear, as a rule, before the perfusion of the pituitary extract had been shut off; in short, the result was only momentary.

The results with an extract prepared from the infundibular body were very different. On perfusion of a two per cent solution the beats were less frequent and decreased in amplitude, and the tonus fell a little and then sometimes rose considerably. No after effects were observed, and a second dose did not appear to be as potent as the first.

The infundibulum seemed to be the more powerful of the two bodies, for the heart was affected by a much smaller dose (two per cent) than in the case of pituitary portion, which usually required to be made much stronger (ten per cent) before any change could be detected in the character of the apical contractions.

Orchitic extract. — In 1889 Brown-Séquard[3] published the results of a series of experiments with orchitic extract upon himself. He declared that the power of his voluntary muscles was considerably increased by the injections made. The increase of power he considered to be not the result of a simple stimulus, as the effect continued too long for this, but rather to be of a dynamogenic nature. That this substance also exercises, in some states, a beneficial influence on the blood has been shown by Hénocque,[1] who found that in phthisical patients a permanent increase in the quantity of hæmoglobin takes place. Poehl[2] prepared from the testicles a substance he calls "spermine," in which he finds high oxidizing powers. De Tarchanoff[3] found that the administration of "spermine" rendered the heart resistent to the effects of chloroform. Brown-Séquard[4] pointed out however that neither this substance nor the Charcot-Naumann crystals are the active principle of orchitic extract. On the isolated cat's heart Hedbom[5] noted that orchitic extract produces increased frequency and force of the cardiac contractions; the effect lasts for some time.

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[1] HALLER: Morphologisches Jahrbuch, 1896, xxv, p. 31.

[2] BERKLEY: The Johns Hopkins hospital reports, 1895, iv, p. 285.

[3] BROWN-SÉQUARD: Archives de physiologie, 1889, xxi, p. 651.

In my own experience the result of perfusing orchitic extract has varied according to the dose. A small dose, four per cent, of glycerine extract, simply caused an increase in the force of the apical contraction with no visible change in tonus or rhythm. A larger dose, on the other hand, caused a marked fall in tonus, and a few moments later a quickening in the rhythm; the contractions were also slightly increased in force.

The changes produced by a small dose made their appearance almost immediately and disappeared on resuming the normal perfusion. With a stronger dose, ten per cent, the fall in tonus occurred with the beginning of orchitic perfusion and passed off as soon as normal blood was perfused, while the increase in frequency and force lasted for some time after.

Liver. — The liver apparently contains nothing soluble in glycerine or saline solution that affects the musculature of the heart. In six experiments the perfusion of saline solutions up to thirty per cent strength produced no change in force or frequency and caused but a slight fall in tonus, which passed away as soon as the apex was supplied with the normal perfusion fluid.

Pancreas. — That the pancreas secretes some material necessary to the normal functions of the body is of course well known. Notwithstanding this the perfusion of the apex with blood containing less than twenty per cent of the saline extract gave little or no result in six experiments. But when the perfusion fluid contained twenty per cent or more of the extract the contractions were lessened in frequency and a fall in tonus was produced. The apex recovered shortly after resuming the normal perfusion.

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[1] HÉNOCQUE: Archives de physiologie, 1892, xxiv, p. 45.

[2] POEHL: Berliner klinische Wochenschrift, 1893, Nr. 36.

[3] DE TARCHANOFF: Archives ital. de biologie, 1895, xxii, p. xxxix.

[4] BROWN-SÉQUARD: Archives de physiologie, 1891, xxiii, p. 400.

[5] HEDBOM: Skandinavisches Archiv für Physiologie, 1898, viii, p. 147.

Submaxillary gland. — An extract of the submaxillary gland of the same strength and character as the pancreas extract was perfused with a very similar result, the only difference being that the fall in tonus was not quite so well marked. Hedbom[1] found the action of this gland to be tonic in its nature on the whole isolated cat's heart. In six experiments I failed to find anything but a depressing effect in these two substances.

Spleen. — That the spleen has some sort of influence on the work of the pancreas was pointed out by Schiff,[2] in 1862, and more recently Gachet and Pachon[3] state that the internal secretion of the spleen modifies the amylolytic secretion of the pancreas. Oliver and Schäfer[4] find that an extract of the substance of the spleen injected into an animal produces a fall in blood pressure soon followed by a rise and then a slow gradual return to the normal. Hedbom[5] observed that the splenic extract increases the tonus of the heart muscle and tends to regulate the rhythm.

In the present investigation a splenic glycerine extract of ten per cent strength produced a slight fall in tonus and slightly increased the frequency and strength of the contractions. It also appeared to make the rhythm more regular. On returning to normal perfusion the contractions became still more forcible, but this effect gradually passed off and the contractions resumed their normal appearance. The above results were, however, far from constant; in about forty per cent of the experiments with this extract little or no change in the character of the contraction was noticed.

The results obtained with the four organs thus far considered, liver, pancreas, submaxillary gland, and spleen, are hardly as satisfactory as could be wished. To produce an effect the extracts had to be given in very large doses, and even then the result was not always well marked.

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[1] Hedbom: *loc. cit.*

[2] Schiff: quoted by Gley: Archives de physiologie, 1892, xxiv, p. 391.

[3] Gachet and Pachon: Archives de physiologie, 1898, xxx, p. 364.

[4] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 278.

[5] Hedbom: *loc. cit.*

Kidney. — That the kidney has an internal secretion was pointed out by Brown-Séquard,[1] and the same observer in collaboration with d'Arsonval[2] showed that injections of kidney extract, prepared in a similar manner to orchitic extract, is of benefit when the internal secretion of the kidney is deficient, either from extirpation or extensive disease of the organ. Meyer,[3] from observations made mainly on the respiratory system, confirmed this statement. These observers also showed that although the urine may be completely suppressed for some time, fatal results do not by any means immediately ensue, in fact the suppression may exist for weeks; on the other hand death rapidly follows the extirpation of both kidneys; finally, in these cases of double extirpation animals will live for a much longer time than otherwise if given injections of kidney extract. Bradford's[4] experiments also demonstrate the importance of the kidney in vital processes other than the mere excretion of urine. In short, it appears from these investigators that the kidney is not merely a simple remover of waste products, but that it also produces a substance stimulating to anabolic processes and obstructive to tissue disintegration.

Tigerstedt and Bergman[5] more recently have shown that the injection of kidney extract into the circulation causes a slight fall in the blood pressure followed by a considerable rise. They did not find that the kidney extracts much affected the heart isolated by Langendorff's method.

In these experiments, done on the hearts of two animals and with two apical preparations from each heart, there was found a decided alteration in the character of the contraction. Perfusion of solutions from two to eight per cent strength caused a drop in tonus and a slight slowing of the rate of contraction (Fig. 3). The slowing seen in Fig. 3 is more prominent in this tracing than in any other obtained, although it was present in all. As may be observed, it was soon succeeded by much more forcible contractions of about the normal frequency. On perfusion with normal blood the apex quickly returned to its usual beat.

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[1] BROWN-SÉQUARD: Archives de physiologie, 1893, xxv, p. 778.

[2] BROWN-SÉQUARD and D'ARSONVAL: Comptes rendus de l'academie des sciences, 1892, cxiv, p. 400.

[3] MEYER: Archives de physiologie, 1893, xxv, 760; and 1894, xxvi, p. 179.

[4] BRADFORD: Journal of physiology, 1891, xii, p. xviii.

[5] TIGERSTEDT and BERGMAN: Skandinavisches Archiv für Physiologie, 1898, viii, p. 223.

Perfusion of urea dissolved in the normal saline solution was also tried, and in amounts up to 2.5 per cent was found to act invariably as an excitant: the contractions were augmented and accelerated but irregular in character; a slight rise in tonus took place.

Thyroid.[1] — On injecting an extract of the thyroid gland Schäfer[2] obtained a fall in blood pressure but no change in the heart-beat. Cunningham[3] on the other hand observed not only a fall in blood pressure but also an increased force of contraction. Hutchinson[4] found that the extract had no effect on the heart, but obtained a fall in blood pressure. The colloid material of the gland gave him negative results, — only the salts were active. Artificial compounds of iodine and albumin are without effect according to this observer. Vomossy and Vas[5] and Kobert[6] declare that thyro-iodine has no effect on the heart, and Schuster[7] finds that even large doses cause no alteration in the pulse in man. Mossé[8] found by means of the ergograph that the work done by the voluntary muscles was increased by the administration of thyro-iodine; the onset of fatigue appeared to be postponed. Cyon[9] asserts that the intravenous injection of thyro-iodine augments the excitability of the vagi, while the injection of iodine exerts an opposite effect: *i. e.*, increases the excitability of the sympathetic, quickens the heart, and raises the blood pressure. He therefore concludes that thyro-iodine is a direct antagonist of iodine.

In my experiments, the perfusion of a fluid containing three per cent of a glycerine extract of the thyroid gland considerably augmented the force and slightly quickened the rate of contraction, but the effect gradually wore off (Fig. 4). It was also noticed that the extract appeared to exert a regulating influence on the rhythm. Small doses of saline extract gave exactly similar results. Large doses of saline extract, 15 to 20 per cent, had in most respects a contrary effect; there was a marked fall in tonus and the contractions became considerably less forcible. But the rhythm, as in the case of the weaker extracts, appeared to be more regular during the perfusion than either before or after (Fig. 5). Continued perfusion of the stronger extracts would eventually paralyze the apex.

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[1] Reported in abstract to the Boston Society of Medical Sciences and published in their journal, 1898, iii, p. 58.

[2] SCHÄFER: British medical journal, 1895, p. 343.

[3] CUNNINGHAM: Journal of experimental medicine, 1898, iii, p. 148.

[4] HUTCHINSON: Journal of physiology, 1898, xxiii, p. 178.

[5] VOMOSSY and VAS: Münchener medicinische Wochenschrift, 1897, Nr. 25.

[6] KOBERT: Verhandlungen des 14ten Congresses für innere Medicin, 1896, p. 153.

[7] SCHUSTER: Wiener medicinische Wochenschrift, 1896, p. 379.

[8] MOSSÉ: Archives de physiologie, 1898, xxx, p. 742.

[9] CYON: Archiv f. d. ges. Physiol., 1898, lxx, p. 126.

Thyro-iodine, prepared according to Baumann's method[1] and dissolved by the aid of sodium bicarbonate, when mixed in small or large amounts with the perfusion fluid produced effects on the apex exactly similar to those caused by glycerine or saline extracts.

In order to test the regulating influence of the thyroid extract on the rhythm, the apex was perfused while the contractions were extremely irregular. The result was an immediate slight fall in tonus. The beat then became a little more forcible and more regular, and, as the thyroid perfusion was stopped and the normal perfusion resumed, the ventricular strip beat in excellent rhythm and slightly quicker than during the thyroid perfusion.

A solution of iodine (0.05 per cent) dissolved in sodium chloride solution (0.8 per cent) was also tried, but the results were entirely different from those obtained with either thyro-iodine or extracts of the gland. At first the beats were increased in size, but they gradually fell to their normal before the cessation of the perfusion; a slight rise in tonus took place; the apex became very irregular in its contractions, and the irregularity continued a considerable time after resuming normal perfusion.

THE ACTION OF THE CULTURES AND CULTURE FILTRATES OF BACTERIA.

Several observers have obtained from toxines direct results on the heart, but most of these were due to degenerative changes and some time necessarily elapsed before the effects became marked. Charrin[2] produced by the injections of toxines such degenerative changes in the heart that heart failure took place. This same observer, with Bardier,[3] in a series of experiments made upon frogs, found that six hours after an intraperitoneal injection of 1 c.c. of diphtheria toxine, the heart was slowed; alteration in the temperature did not modify this action. The effect, they remark, may be due to stimulation of the vasomotor centre. Enriquez and Hallion[1] assert that the toxine of diphtheria paralyzes the inhibitory nerves of the heart while the accelerator nerves preserve their action. Roger[2] found, in frogs, that the bacillus diphtheriae lessened both the force and frequency of contraction. Large doses were required to produce the result, which even at the best was not well marked. Another organism, which he speaks of as bacillus septicus putidus, possessed this depressant action in a powerful degree. Sharp[3] removed the frog's heart and passed through it the products of a culture tube of the Klebs-Loeffler bacillus mixed with his perfusion fluid; a prolonged diastole and subsequent heart failure was the result.

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[1] BAUMANN and ROOS: Zeitschr. f. physiol. Chemie, 1896, xxi, p. 481. For the preparation of the thyro-iodine used in these experiments I am indebted to the kindness of Dr. Alfred W. Balch, of the Pharmacological Laboratory, Harvard Medical School. The thyro-iodine was prepared from perfectly fresh glands and used at once.

[2] CHARRIN: C. r. de la société de biologie, 1896, p. 867.

[3] CHARRIN and BARDIER: Archives de physiologie, 1897, xxix, p. 554.

The following bacteria and their filtrates were used in the present experiments: Staphylococci pyogenes aureus, anthrax, coli communis, pyocyaneus, typhoid, megatherium, cholera spirillum, capsulatus, diphtheria, ramosus, prodigiosus, and glanders.[4] All of the above bacteria, pathogenic and non-pathogenic, when perfused unfiltered, exercised a depressant action on the apical preparation, almost immediately slowing the rate and weakening the force of the contractions. The result seemed to vary according to the size of the bacterium; cultures of large bacteria giving the most pronounced effect, while smaller ones did not produce so great a change. Fig. 6 shows the abrupt diminution in force and frequency of the contractions produced by the perfusion of a culture of anthrax. The quick recovery of the apex is remarkable. If the perfusion of the bacteria is carried on too long, however, either with pathogenic or non-pathogenic organisms, the apex very often fails to recover its normal, regular contractions.

The action of the filtrate on the apex was entirely different in several particulars from that of the bacteria; first, no results were obtained, in doses up to thirty per cent, from filtrates of non-pathogenic bacteria; secondly, the action obtained from the perfusion of pathogenic filtrates came on slowly, was depressant in its character, and passed off slowly when normal perfusion was resumed. It was necessary to give the filtrate in large doses to produce these effects, and when the perfusion was carried on for some time the apex quickly lost its contractile power (Fig. 7).

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[1] Enriquez and Hallion: Archives de physiologie, 1898, xxx, p 393.

[2] Roger: C. r. de la société de biologie, 1893, p. 175.

[3] Sharp: Journal of anatomy, 1897, xxxi, p. 199.

[4] I am indebted to the kindness of Dr. A. K. Stone of the Bacteriological Laboratory, Harvard Medical School, for the preparation of the cultures, toxines, and filtrates.

The conclusion I am forced to draw from the above phenomena is that the action of the bacteria on the contracting apex is mainly mechanical. Combined with this, in the case of pathogenic bacteria, is the depressant influence of their filtrates. The pathogenic filtrates are purely depressant; their action possibly was partly hidden by the stimulating properties of the serum proteids contained in the solution.

Very powerful fresh toxines of tetanus and diphtheria were also employed. The latter, when perfused in doses of 25 per cent, slowed the rate but did not greatly alter the force of beat; the tetanus toxine on the other hand was absolutely without effect, even in solutions containing 40 per cent.[1]

This research was undertaken at the suggestion of Professor W. T. Porter, and I am greatly indebted to his kind criticism of the results obtained.

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[1] For these I must thank Professor Theobald Smith, of the Laboratory of Comparative Pathology, Harvard University.

THE FORMATION OF MELANINS OR MELANIN-LIKE PIGMENTS FROM PROTEID SUBSTANCES.

By R. H. CHITTENDEN and ALICE H. ALBRO, Ph. D.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE brownish black animal pigments, collectively known as melanins, occurring normally and pathologically in the body, are characterized as a class by a somewhat high content of carbon, a relatively low content of nitrogen, and a variable, though usually high, percentage of sulphur. The presence of the latter element in very appreciable amounts constitutes one of the reasons for the belief that these substances have their origin in some proteid antecedent, while the absence of iron, in most cases, excludes the view that they originate from the blood pigment. In a recent contribution by Schmiedeberg[1] to the chemical composition and nature of melanins, emphasis is laid upon the possible origin of these substances in antialbumid. This view is seemingly based upon the partial resemblance in chemical composition between the latter substance and the melanins; a resemblance which is indeed somewhat striking. As a cleavage product of the proteids, antialbumid is characterized by a comparatively high content of carbon and a low content of nitrogen, and Schmiedeberg concludes that melanin may result from antialbumid by a process of hydrolytic cleavage comparable to the method by which the latter body results from albumin itself. He further points out that the sulphur of the antialbumid may remain with the melanin, thus accounting for the large amount of this element usually found, while ammonia and water are split off according to the following equation: —

$$\underset{\textit{Antialbumid.}}{C_{120}H_{187}N_{27}SO_{37}} = \underset{\textit{Melanin.}}{C_{120}H_{114}N_{8}SO_{29}} + 19\,NH_3 + 8\,H_2O.$$

This formula for antialbumid is based upon an analysis of antialbumid prepared by Kühne and Chittenden[2] from serum-albumin, while the formula for the melanin is based upon the analysis of a product (melanoidic acid) prepared by Schmiedeberg[1] by boiling serum-albumin with 25 per cent hydrochloric acid for twelve hours. Schmiedeberg likewise prepared a melanoidic acid from the fibrinoses contained in Witte's "pepton" by heating these proteoses with phosphoric acid for two months. Ascribing to the proteoses present in Witte's "pepton" a formula with 102 atoms of carbon, he formulates the production of the melanin as follows:

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[1] SCHMIEDEBERG: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 65.

[2] KÜHNE and CHITTENDEN: Zeitschrift für Biologie, 1883, xix, p. 176.

$$\underset{\textit{Fibrinose.}}{C_{102}H_{150}N_{30}SO_{31}} + 18\ H_2O = \underset{\textit{Melanin.}}{C_{102}H_{98}N_{12}SO_{32}} + 18\ NH_3 + 17\ H_2O.$$

It is to be observed that these two artificial melanins or melanoidic acids differ from each other by nearly 6 per cent of carbon and 3 per cent of nitrogen, and that sulphur was determined in only one of the products, the amount found being 0.96 per cent. While the data may be considered as perhaps hardly sufficient to justify such definite expressions as the above formulæ, the general trend of the argument is exceedingly interesting and important. Any one who has worked much with the proteids is aware how readily these substances yield dark colored solutions on boiling with dilute mineral acids; a reaction not due to oxidation alone since it takes place readily even in the presence of reducing agents. It is thus evident that melanin-like substances may be formed from proteids by simple hydrolytic cleavage, and consequently the question at once arises how far the nature of the mother substance modifies the character of the resultant pigment. Further, is one proteid better adapted for the artificial production of a melanin than another proteid? How closely do the artificial melanins resemble in composition and general characters the natural pigments of this class? And lastly, how far can reactions of this kind be accepted as indicating the mode of formation of the natural pigments common to the body in health and disease? Some of these questions we have endeavored to answer by the following experimental work.

Formation of Melanin from Antialbumid. — In view of the somewhat noticeable parallelism in composition between antialbumid and the melanins, attention was first directed to the possible formation of a pigment of this class by hydrolysis of antialbumid. For this purpose a pure antialbumid of known composition was necessary, and since the latter body is prone to slight alteration by too vigorous hydrolysis particular attention was paid to the conditions attending its formation. As the mother substance in the preparation of antialbumid, thoroughly washed, coagulated egg-albumin was employed. In the hydrolysis, 2600 grams of the moist coagulum, pressed as dry as possible, were heated with 7 litres of 3.0 per cent sulphuric acid in a large flask, filled quite to the neck to diminish oxidation, at 100° C. (in a large Arnold sterilizer) for 10 hours. The gelatinous mass of impure antialbumid was thrown upon filters, allowed to drain, and after some washing with water, was transferred to a flask and heated with 3.5 per cent sulphuric acid for 10 hours, after which it was again filtered off and washed with water — by decantation and otherwise — until the washings were nearly or quite free from acid reaction. To remove any traces of unaltered proteid possibly present, as well as other impurities, the antialbumid was next warmed at 38° C. for 30 hours with an active solution of pepsin-hydrochloric acid (0.2 per cent HCl), the soluble products removed by filtration, and the residual antialbumid washed with water until the washings gave no reaction with the biuret test, nor with silver nitrate for chlorine. The washing was facilitated, after the bulk of the soluble matter had been removed, by carefully adding to the antialbumid suspended in water sufficient dilute solution of potassium hydroxide to make the mixture quite neutral to test paper, thus aiding the removal of any loosely combined sulphuric acid. Finally it was washed with weak and strong alcohol and lastly with ether. When dry, the finely powdered substance was repeatedly boiled with distilled water to insure the complete removal of any adherent soluble salts.

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[1] SCHMIEDEBERG: *loc. cit.*, p. 66.

Analysis of a sample of this product dried at 110° C. until of constant weight gave the following results:

I. 0.3397 gram substance gave 0.2098 gram H_2O = 6.86 per cent H and 0.6654 gram CO_2 = 53.45 per cent C.
II. 0.3439 gram substance gave 0.2114 gram H_2O = 6.83 per cent H and 0.6719 gram CO_2 = 53.29 per cent C.
III. 0.2259 gram substance gave by the Kjeldahl method 0.03054 gram N = 13.52 per cent N.
IV. 0.2502 gram substance gave 0.03429 gram N = 13.70 per cent N.
V. 0.6210 gram substance gave by fusion with NaOH[1] and KNO_3 0.1001 gram $BaSO_4$ = 2.21 per cent S.
VI. 1.0297 grams substance gave 0.0031 gram ash = 0.30 per cent.

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[1] Pure NaOH prepared from metallic sodium and free from sulphur. The fusion was made over an alcohol lamp.

Percentage composition of the ash-free antialbumid.

	I	II	III	IV	V	*Average*
C	53.59	53.45	....	....	....	53.52
H	6.86	6.83	....	....	....	6.84
N	....	....	13.56	13.74	....	13.65
S	....	....	....	....	2.22	2.22
O	....	....	....	....	....	23.77
						100.00

Comparison of the following figures shows the relationship in composition between the antialbumid and the mother substance from which it was derived.

	Coagulated egg-albumin.[1]	*Antialbumid.*
C	52.18	53.52
H	6.93	6.84
N	15.81	13.65
S	1.87	2.22
O	23.21	23.77

Most conspicuous is the marked loss of nitrogen and the corresponding rise in carbon; results which accord more or less closely with the earlier data obtained by Kühne and Chittenden.[2] Also noticeable is the somewhat larger percentage of sulphur present in antialbumid. A large amount of this sulphur exists loosely combined in the antialbumid molecule, and the question at once arises whether this loosely combined sulphur can be driven off by continued hydrolysis with dilute sulphuric acid. This point was tested by taking a portion of the antialbumid prepared as above and heating it further with 3 per cent sulphuric acid. After exposure at 100° C. in the sterilizer for 6 hours the acid fluid was found to yield a strong reaction for loosely combined sulphur, while the residue of antialbumid gave an equally marked reaction for loosely combined sulphur. The acid fluid was therefore filtered off, the antialbumid washed with water, and again heated for some hours with fresh 3 per cent sulphuric acid. This process was repeated until the aggregate period of heating had reached 38 hours, at the end of which time the last acid fluid was found free from loosely combined sulphur. The antialbumid remaining, however, still gave a striking reaction for sulphur with potassium hydroxide and plumbic acetate, thus showing that the loosely combined sulphur contained in the antialbumid molecule cannot be removed entirely by this method of hydrolysis. Especially noteworthy was the continued shrinkage of the antialbumid during this long process of heating with the dilute acid, until at the termination of the treatment the substance was greatly diminished in bulk; a fact which accords with Schützenberger's[1] original observations that this substance, when heated continuously with dilute acid, slowly but progressively disappears. Somewhat noticeable, however, is the fact that such portion of the antialbumid as does resist this long continued action of the dilute sulphuric acid is not widely different in composition from the original antialbumid. Thus, analysis of the product remaining after the 38 hours' heating gave the following results:

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[1] Chittenden and Bolton: Studies in physiological chemistry, Yale University, 1887, ii, p. 130.

[2] Kühne and Chittenden: Zeitschrift für Biologie, 1883, xix, p. 176.

I. 0.1971 gram substance gave 0.1245 gram H_2O = 7.01 per cent H and 0.3937 gram CO_2 = 54.48 per cent C.
II. 0.3016 gram substance gave 0.6035 gram CO_2 = 54.58 per cent C.
III. 0.2972 gram substance gave by the Kjeldahl method 0.04113 gram N = 13.84 per cent N.
IV. 0.2093 gram substance gave 0.02860 gram N = 13.66 per cent N.
V. 0.5487 gram substance gave by fusion with NaOH and KNO_3 0.1058 gram $BaSO_4$ = 2.63 per cent S.
VI. 0.4770 gram substance gave 0.0015 gram ash = 0.31 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C	54.65	54.75	....	....	....	54.70
H	7.02	....	....	....	....	7.02
N	....	....	13.88	13.71	....	13.79
S	....	....	....	....	2.64	2.64
O	....	....	....	....	....	21.85
						100.00

From these data it is evident that the antialbumid which has resisted this long continued treatment with dilute sulphuric acid has gained somewhat in its content of sulphur and still more in its content of carbon. Nitrogen, on the contrary, remains essentially the same. This tendency of an antialbumid, on hydrolysis, to grow richer in carbon usually at the expense of the nitrogen is exceedingly characteristic. Thus, it was found by Kühne and Chittenden[2] on subjecting a sample of antialbumid (from egg-albumin) to the action of an alkaline solution of trypsin that the antialbumid remaining undigested contained 55.54 per cent of carbon when dry, while the original antialbumid contained 53.79 per cent of carbon. Similarly, an antialbumid, prepared from serum-albumin, containing 54.51 per cent of carbon and 14.31 per cent of nitrogen, on being subjected to the action of trypsin in an alkaline medium yielded an undigested residue containing 58.09 per cent of carbon and 12.61 per cent of nitrogen; results which clearly testify to the innate tendency of antialbumid under suitable conditions to undergo hydrolysis and presumably also cleavage with formation of a more insoluble and resistant body, of the antialbumid type, with a higher content of carbon.

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[1] SCHÜTZENBERGER: Bulletin de la société chimique de Paris, 1875, xxiii, p. 161.

[2] KÜHNE and CHITTENDEN: *loc. cit.*

With a view to the possible preparation of a melanin, 30 grams of pure dry antialbumid (the first sample analyzed) were placed in a flask with 300 c.c. of 10 per cent sulphuric acid, the flask connected with an inverted Liebig's condenser, and the mixture boiled directly over a flame for 79 hours. When first heated, the acid was almost colorless, but as decomposition progressed the color changed first to purple and finally to jet black. Early in the course of the heating a light film, shown later to be composed of fatty acids, was deposited on the sides of the condenser, and the odor of volatile fatty acids was very distinct.[1] As the boiling progressed, bright yellow crystals in rosettes were seen, both within the condenser and clinging to the neck and sides of the flask. These crystals were eventually collected and tested. They burned with a blue flame, forming acid fumes; they were insoluble in water, alcohol, and ether, but rapidly soluble in carbon disulphide. From the latter fluid they recrystallized in the characteristic rhombic octahedra of native sulphur.

At the conclusion of the 79 hours of heating, and after the acid fluid had cooled, the mixture was filtered through paper, leaving a residue of black amorphous matter, while the clear filtrate was quite black in color. Obviously, the fluid contained considerable black pigment in solution, but owing to the large admixture of leucin, tyrosin, and other substances present, attempts to isolate the pure pigment from the solution were not very successful. Attention was therefore directed to the insoluble pigment on the filter paper. This was washed with water until free from acid, and then with alcohol and ether, in all of which it was insoluble. As the pigment was found to be extremely soluble in weak alkalies, the precipitate was treated with a very little 0.2 per cent potassium hydroxide solution, in which it quickly dissolved, leaving a small slate-colored residue (too small to identify) which was readily washed free from the melanin-like substance. Although the volume of the weak alkaline fluid with the washings now amounted to full 500 c.c. the clear fluid was jet black in color and absolutely opaque to light. On neutralization of this fluid with dilute acetic acid the melanin was reprecipitated in large dark flocks, leaving a colorless transparent fluid. The pigment was filtered off, washed entirely free from salts with water, and thoroughly extracted with alcohol and ether, after which it was dried to a constant weight at 105° C. When dry the preparation weighed 1.498 grams, equal to practically 5 per cent of the original antialbumid.

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[1] See R. COHN: Zeitschr. f. physiol. Chem., 1896, xxii, p. 153.

On analysis this substance gave the following results:

I. 0.3598 gram substance gave 0.2249 gram H_2O = 6.94 per cent H and 0.7158 gram CO_2 = 54.26 per cent C.
II. 0.2044 gram substance gave by the Kjeldahl method 0.02453 gram N = 12.00 per cent N.
III. 0.2273 gram substance gave 0.02733 gram N = 12.00 per cent N.
IV. 0.3691 gram substance gave by fusion with NaOH and KNO_3 0.2063 gram $BaSO_4$ = 7.70 per cent S.

Percentage composition of the melanin.[1]

	I	II	III	IV	*Average*
C	54.26	....	....	....	54.26
H	6.94	12.00	12.00	....	6.94
N	....	....	....	....	12.00
S.	....	....	....	7.7	7.70

A second decomposition, using a larger amount of the same antialbumid — 61 grams — was now attempted under exactly the same conditions as before, except that the acid mixture was boiled for 110 hours. Fatty acids and free sulphur were detected as before, while the same separation of a black pigment took place. The latter was collected on two filters and the precipitates washed as already described, after which the larger precipitate was dissolved in water containing a little ammonia, the clear filtered solution precipitated by neutralization with 0.2 per cent hydrochloric acid, and the precipitate thoroughly washed with water, alcohol, and ether. When dry, it weighed 2.728 grams. The smaller portion of crude melanin was dissolved in 0.2 per cent solution of potassium hydroxide and the clear filtered solution precipitated with dilute hydrochloric acid, etc. as just described. When dry, this preparation weighed 0.675 gram. Hence, the total yield of melanin insoluble in the 10 per cent sulphuric acid amounted to 3.403 grams, or 5.5 per cent of the original antialbumid.

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[1] The ash could not be determined with any accuracy owing to the scarcity of substance, but the small amount of ash left in the platinum tray in the determination of carbon made it quite clear that not more than a small fraction of one per cent could be present.

The melanin formed in this decomposition — the portion which had been purified by solution in dilute ammonia — gave on analysis the following results, after being dried at 105° C.

I. 0.3140 gram substance gave 0.2130 gram H_2O = 7.53 per cent H and 0.6631 gram CO_2 = 57.60 per cent C.
II. 0.2012 gram substance gave 0.1300 gram H_2O = 7.17 per cent H and 0.4267 gram CO_2 = 57.85 per cent C.
III. 0.2247 gram substance gave by the Kjeldahl method 0.02671 gram N = 11.88 per cent N.
IV. 0.2245 gram substance gave 0.02656 gram N = 11.83 per cent N.
V. 0.5479 gram substance gave by fusion with NaOH and KNO_3 0.1749 gram $BaSO_4$ = 4.39 per cent S.
VI. 0.4050 gram substance gave by fusion with NaOH and KNO_3 0.1258 gram $BaSO_4$ = 4.27 per cent S.
VII. 0.4616 gram substance gave 0.0025 gram ash = 0.54 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	VI	*Average*
C	57.91	58.16	....	....	....	....	58.05
H	7.57	7.21	....	....	....	....	7.39
N	....	....	11.94	11.90	...	....	11.92
S	....	....	....	....	4.41	4.29	4.35
O	....	....	....	....	....	....	18.29
							100.00

The smaller fraction of this same melanin which had been purified by solution in 0.2 per cent potassium hydroxide contained the following percentages of nitrogen and sulphur:

0.2046 gram substance gave by the Kjeldahl method 0.02379 gram N = 11.63 per cent N.
0.4070 gram substance gave by fusion with NaOH and KNO_3 0.1272 gram $BaSO_4$ = 4.29 per cent S.

These results seemingly show that the sulphur and nitrogen content of the melanin is not materially affected by the character of the alkali used to dissolve it; *i. e.* a fixed alkali, when dilute, causes no marked withdrawal of sulphur or nitrogen. Natural pigment from hair or epidermis when treated with 10 per cent solution of potassium hydroxide, and then reprecipitated by acid is liable to lose both nitrogen and sulphur,[1] but whether this is due to a change in the pigment itself produced by the strong alkali, or whether due to the withdrawal of contaminating substances derived from the original pigmentary granules, is uncertain.

Let us compare now the composition of our artificial melanins with that of the antialbumid from which they were derived.

	Antialbumid.	*Melanin.* 79 hours boiling.	*Melanin.* 110 hours boiling.	*Antialbumid.*[2]
C	53.52	54.26	58.05	54.70
H	6.84	6.94	7.39	7.02
N	13.65	12.00	11.92	13.79
S	2.22	7.70	4.35	2.64

The results seemingly justify the conclusion that these melanins formed from antialbumid originate not by simple hydrolysis, but by a process of hydrolytic cleavage, the pigment holding the position of a cleavage residue, the exact composition of which depends upon the extent or intensity of the cleavage process. Further, it is evident that this melanin-like residue, *i. e.* the true pigment, is either contaminated by some substance or substances, which accounts for the marked variation in composition, or else that under the term *melanins* we have a class of related bodies more or less alike in their physical properties but unlike in chemical composition. Certainly, our knowledge regarding the composition of the natural melanins lends favor to the latter view, for it is a well-known fact that certain melanins are exceedingly rich in sulphur (10 per cent), like the phymatorhusin of Berdez and Nencki,[3] while others, like the choroidal pigment,[4] are entirely free from sulphur. Again, while the majority of these pigments contain practically no iron at all, others contain 0.5 per cent of this element.[5] Hence there may be justification for the suggestion made by Brandl and Pfeiffer [6] that the melanins should be divided into groups to be designated as ferro-melanins, sulpho-melanins, etc. The melanins which we have prepared from antialbumid are practically free from iron, the ash containing only the merest trace of this element. Somewhat suggestive is the fact that in both of our experiments with antialbumid, in spite of the difference in the length of the hydrolytic process, the yield of melanin in both cases amounted to about 5 per cent.

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[1] See ABEL and DAVIS: Journal of experimental medicine, 1896, i, p. 391. Also, M. NENCKI and N. SIEBER: Archiv f. exper. Pathol. u. Pharmakol., 1887, xxiv, p. 17.

[2] After 38 hours' heating with 3 per cent sulphuric acid.

[3] BERDEZ and M. NENCKI: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 346.

[4] SIEBER: *Ibid.*, 1886, xx, p. 362.

[5] See BRANDL and PFEIFFER: Zeitschrift für Biologie, 1890, xxvi, p. 348.

[6] *Loc. cit.*

Formation of melanin from so-called hemipeptone. — Having demonstrated the possibility of preparing a melanin-like substance by the hydrolysis of antialbumid, the question naturally arose whether bodies of the so-called hemi class will likewise yield a melanin by hydrolysis. To test this point, so-called hemipeptone formed by the hydrolysis of coagulated egg-albumin with 3 per cent sulphuric acid was employed. The peptone was prepared by using the acid fluid, resulting in the formation of antialbumid. The fluid was neutralized with ammonia, the filtered solution concentrated and the albumoses separated collectively by saturation of the fluid with ammonium sulphate, after the method suggested by Kühne.[1] After complete removal of the albumoses, the excess of ammonium sulphate was separated by alternate concentration and crystallization, after which the last portions of the salt were removed by treatment with barium carbonate.[2] The filtrate, freed from all traces of barium by cautious addition of dilute sulphuric acid, was concentrated to a syrup and the peptone precipitated by alcohol. The product was purified somewhat by repeated boiling with alcohol and thorough extraction with ether.

In the production of a melanin 90 grams of this hemipeptone were boiled for 98 hours with 2 litres of 10 per cent sulphuric acid. The fluid rapidly became black in color, and gradually there occurred a small separation of a black pigment. There was also noticeable, especially toward the end of the boiling, the odor of volatile fatty acids, and some slight crystallization of fatty acids upon the walls of the condenser could be detected likewise. There was however no evidence of the splitting off of free sulphur, so conspicuous with antialbumid. At the expiration of the 98 hours, the fluid was cooled, and the melanin-like substance collected by filtration. The very dark filtrate on standing and concentrating continued to deposit small portions of black pigment, the process being repeated until several fractions were obtained. The fluid, however, still gave evidence of the presence of considerable pigment in solution. The united fractions of pigment were washed thoroughly with water, then dissolved in 0.2 per cent solution of potassium hydroxide and the filtered fluid precipitated by neutralization with 0.2 per cent hydrochloric acid. The pigment was next washed free from soluble salts with water and extracted with alcohol and ether. When freshly precipitated this melanin, unlike that prepared from antialbumid, was somewhat soluble in water, but if allowed to dry upon the filter it could be washed with water without loss. The amount of pigment obtained was 0.9 gram, equal to 1.0 per cent of the original hemipeptone.

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[1] KÜHNE: Zeitschrift für Biologie, 1892, xxix, p. 1.

[2] See CHITTENDEN, MENDEL, and HENDERSON: This journal, 1899, ii, p. 173.

When dried at 105° C. until of constant weight, it gave on analysis the following results:

I. 0.2435 gram substance gave 0.0815 gram $H_2O = 3.72$ per cent H and 0.5156 gram $CO_2 = 57.75$ per cent C.

II. 0.2068 gram substance gave by the Kjeldahl method 0.01986 gram $N = 9.60$ per cent N.

III. 0.2796 gram substance gave by fusion with NaOH and KNO_3 0.0569 gram $BaSO_4$ $= 2.79$ per cent S.

IV. 0.2435 gram substance gave 0.0192 gram ash = 6.11 per cent.

Percentage composition of the ash-free substance.

	I	II	III	*Average*
C	61.50	....	....	61.50
H	3.97	....	....	3.97
N	....	10.23	....	10.23
S	....	....	2.98	2.98
O	....	....	....	21.32
				100.00

It is thus plainly evident that the melanin obtained from hemipeptone is widely different in chemical composition from the corresponding body obtained from antialbumid. It resembles in its content of carbon the melanin-like body prepared by Schmiedeberg from Witte's "pepton," but differs widely from it in the amount of both nitrogen and sulphur. It would seem that the artificial melanins differ as widely among themselves in composition as the natural melanins obtainable from melanotic tumors, etc., or from normal pigmentary deposits. Certainly the contrast between the melanoidic acid with its 66 per cent of carbon obtained by Schmiedeberg from serum-albumin and the corresponding pigments with 54, 58, and 61 per cent of carbon respectively obtained by us from antialbumid and hemipeptone, is very striking.

Reactions of the melanins. — The pigments formed in the manner described above were practically insoluble in water, although, as stated, the sample obtained from hemipeptone was somewhat soluble when freshly precipitated. They were likewise insoluble in alcohol, ether, chloroform, etc., but readily soluble in exceedingly dilute alkaline fluids. They thus differ in some slight degree from the pigment isolated by Abel and Davis[1] from the negro's skin. Further, these pigments seemingly retain their solubility in dilute alkalies in greater degree than the pigments described by Abel and Davis or the melanoidic acid prepared by Schmiedeberg[2] from serum-albumin.

When dry, the pigments were more or less jet black in color, and when dissolved in dilute alkali they yielded yellowish brown, brown, or black colored solutions according to the degree of concentration. These solutions show no absorption bands when examined before the spectroscope, but absorb a certain amount of light at the violet end of the spectrum. Unlike the melanin obtainable from the negro's skin,[3] our pigments were soluble in glacial acetic acid, but were not precipitable therefrom by potassium ferrocyanide. Alkaline solutions of these artificial melanins were completely bleached by chlorine.

Heated on platinum foil, our products, like the pigments described by Abel and Davis, gave off at first fumes of pyrrol, — tested by a pine-sliver moistened with hydrochloric acid, — but these soon ceased, leaving a coal-black residue very difficult of combustion.

From alkaline solutions, the pigments were precipitated by cupric sulphate, silver nitrate, plumbic acetate, and baryta water. By strong nitric acid the pigments were dissolved, but precipitated again on addition of water.

From these few reactions it is seen that our artificial melanins or melanoidins, to use Schmiedeberg's term, differ only in minor degree from the melanins or pigments obtained by Abel and Davis from the skin and hair of the negro, or from the melanins studied by other observers.

Comparison of the composition of the artificial melanins with that of natural melanins, etc. — Our own results bearing on the chemical

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[1] ABEL and DAVIS: Journal of experimental medicine, 1896, i, p. 386.

[2] SCHMIEDEBERG: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxix, p. 66.

[3] ABEL and DAVIS: *loc. cit.*

ORIGIN OF THE PIGMENT.	AUTHOR.	PERCENTAGE COMPOSITION.				
		C	H	N	S	Fe
Hydrolysis of serum-albumin	Schmiedeberg[1]	66.27	5.49	5.57		
Hydrolysis of Witte's "pepton"	Schmiedeberg[1]	60.34	4.86	8.09	0.96	
Hydrolysis of Antialbumid[2]	Chittenden and Albro	54.26	6.94	12.00	7.70	
Hydrolysis of Antialbumid[3]	Chittenden and Albro	58.05	7.39	11.92	4.35	
Hydrolysis of Hemipeptone[4]	Chittenden and Albro	61.50	3.97	10.23	2.98	
Sarcoma (human) of liver and spleen	Berdez and Nencki[5]	53.58	4.22	10.59	10.13	
Sarcoma of horse, liver and spleen	Berdez and Nencki[6]	53.52	3.92	10.48	2.78	
Human urine, sarcoma	K. A. H. Mörner[7]	55.76	5.95	12.27	9.01	0.2
Tumor, horses' spleen	Miura[8]	54.50	5.06	11.75	2.72	
Human liver, sarcoma	Brandl and Pfeiffer[9]	53.58	4.00	9.91	3.02	0.53
Ox eyes, choroidal pigment	Sieber[10]	59.90	4.61	10.81	0	0
Black and brown hair	Sieber[10]	56.14	7.57	8.50	4.10	
Epidermis of negro	Abel and Davis[11]	53.56	5.11	15.47	2.53	
Hair of negro	Abel and Davis[11]	57.06	5.45	12.87	1.77	

[1] SCHMIEDEBERG: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 67.

[2] 79 hours' boiling with sulphuric acid.

[3] 110 " " "

[4] 98 " " "

[5] BERDEZ and M. NENCKI: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 346.

[6] BERDEZ and M. NENCKI: *Ibid.*, 1887, xxiv, p. 17.

[7] K. A. H. MÖRNER: Zeitschr. f. physiol. Chem., 1887, xi, p. 66.

[8] MIURA: Virchow's Archiv für pathologische Anatomie, 1887, cvii, p. 250.

[9] BRANDL and PFEIFFER: Zeitschrift für Biologie, 1890, xxvi, p. 348.

[10] N. SIEBER: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 362.

[11] ABEL and DAVIS: Journal of experimental medicine, 1896, i, p. 392.

composition of the melanin-like pigments obtainable by decomposition of proteids emphasize the view that these substances are many in number, and that, while having many points in common, they differ widely from each other in composition, owing no doubt in part to variations in the extent or intensity of the hydrolytic cleavage by which they are produced. This we fancy is a far more potent factor than the character of the individual proteid from which they are derived. Thus, in our experiments with antialbumid, the first preparation of melanin resulting from 79 hours' boiling of the proteid with sulphuric acid contained 54 per cent of carbon, while the second preparation resulting from 110 hours' boiling contained 58 per cent of carbon. Further, in the content of sulphur the differences were still more striking. The table on page 303, showing the composition of melanins from various sources, affords evidence of the extent to which these black pigments may differ from each other in chemical composition as well as indicating the extent to which the artificial melanins resemble the natural pigments.

These figures, which are fairly typical of the composition of the various melanins hitherto studied, show quite clearly how widely these bodies may vary in composition; yet throughout the entire list, as well as in many other preparations not tabulated, there is to be found almost invariably a relatively high content of carbon and a low content of nitrogen. Further, in all but the choroidal pigment, sulphur is very conspicuous. These facts, coupled with the inappreciable amounts of iron usually found, strengthen belief in the theory that these pigments, whether formed normally or as a result of pathological conditions, have their origin not in the hæmatin molecule but in proteid matter. Further, we see in the experiments of Schmiedeberg, as well as in our own results, evidence that serum-albumin, proteoses, antialbumid, and hemipeptone may all yield melanin-like substances by simple hydrolytic cleavage: pigments which in composition and reaction differ from the natural pigments no more widely than the latter differ from each other. No two melanins are exactly alike in composition, and the artificial bodies, certainly, are exceedingly prone to vary in composition with variations in the method of preparation; especially, variations in the extent and intensity of the hydrolytic cleavage. In the hydrolytic cleavage induced by boiling acids proteid substances tend to lose nitrogen, partially as ammonia and partially in the form of amido-acids and nitrogenous bases, while the artificial melanins simultaneously formed appear to have their origin in the carbon-rich residue left after the splitting off of these nitrogenous radicles. For this reason, perhaps, antialbumid is especially well adapted to be the mother substance of a melanin. Between antialbumid and the melanins there is a certain recognizable kinship in composition, which renders the formation of a melanin-like pigment from this peculiar form of proteid matter an easy task. It is equally clear, from our experiments, however, that melanin may be formed likewise from such dissimilar proteid substances as so-called hemipeptone, from which we are forced to the conclusion that no one form of proteid matter is the sole antecedent of these peculiar brownish black pigments.

A NOTE ON THE CHOLESTERIN-ESTERS OF BIRDS' BLOOD.

By ERNEST W. BROWN, Ph. B.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE interesting investigations of Hürthle[1] have demonstrated that cholesterin occurs normally in combination with fatty acids in the blood-serum of mammals. It has been pointed out that the failure to recognize these cholesterin-esters has been due to the methods employed in the search for cholesterin. Thus the customary procedure has been to saponify ether extracts of animal tissues and fluids with alcoholic potash for the purpose of transforming any fats present into soaps and in this way permitting a more successful subsequent separation of cholesterin by means of ether.[2] The method obviously precludes the possibility of obtaining cholesterin-esters as such. By avoiding the saponification, however, Hürthle has succeeded in demonstrating the presence of cholesteryl oleate in the blood-serum of the dog, sheep, pig, ox, and horse, and in the lymph obtained from the thoracic duct of the dog. Cholesteryl palmitate was also obtained, although in much smaller quantities; while cholesteryl stearate could not be isolated. The quantities of the cholesterin-esters present in the blood were approximately determined as follows:

	Cholesteryl oleate	*Cholesteryl palmitate*
Horse	0.08 per cent	0.006 per cent
Calf	0.09 "	0.008 "
Dog	0.12–0.22 "	

The percentage of cholesteryl oleate was observed to vary in the dog with the condition of the animal, being increased during hunger.[3]

In his contributions to the chemistry of the blood, Hoppe-Seyler[4] has recorded analyses of the blood of the goose which indicate that cholesterin is present in the serum of this species in quantities approaching those of the ox.

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[1] Hürthle: Zeitschrift f. physiol. Chemie, 1896, xxi, p. 331.

[2] Cf. Hoppe-Seyler: Medicinisch-chemische Untersuchungen, 1866, p. 143.

[3] Cf. Hoppe-Seyler: *loc. cit.*, p. 145; Schulz, Fr. N.: Arch. f. d. ges. Physiol., 1896, lxv, p. 299.

[4] Hoppe-Seyler: *loc. cit.*, p. 145.

In the course of some experiments in this laboratory, on the chemistry of birds' blood, the blood-serum of the hen, turkey, goose, and duck has been examined for cholesterin-esters. The observations made are recorded briefly here, since they verify and extend the investigations of Hürthle, whose methods have been employed, for the most part, in the preparation of the esters. The blood-serum was usually precipated with three volumes of alcohol; after standing, the precipitate was filtered off and extracted with fresh alcohol for two or three days at 40° C. Good results were obtained by keeping the precipitate continually agitated in the warm alcohol by means of a slow current of air. The filtered extract deposited the characteristic small needle crystals of the oleate on standing in the cold, while the surface of the alcohol was usually covered with a slight film having a more or less crystalline character (cholesteryl palmitate). The film was separated as far as possible, and the larger mass of fine needle crystals, often grouped in rosettes, was filtered off, washed with cold alcohol, and dried in vacuo over sulphuric acid. The crystals thus obtained were weighed, in order to afford an approximate idea of the quantity of material obtainable from the various serums; this method, as Hürthle has observed, by no means gives accurate determinations, inasmuch as quite appreciable quantities of the corresponding ester remain in solution in the alcohol used. The precipitated serum residues were usually re-extracted with warm alcohol, and finally treated with alcohol-ether for the separation of the remaining cholesteryl palmitate. The yield of substance in the latter process was always small. The ester preparations obtained were purified by recrystallization from alcohol, until the melting points corresponded with those found by Hürthle. In some cases the composition of the product was further established by an elementary analysis. The substances isolated all gave the cholesterin-like reactions described for them.

Hen serum. — Several preparations of cholesteryl oleate were separated from the serum of hens' blood and purified. They all melted at 43–44° C. An analysis of two products gave the following results:

Analysis of Cholesteryl oleate

	Preparation I	Preparation II	Hürthle's average	$C_{44}H_{76}O_2$: Theory
	Per cent	Per cent	Per cent	Per cent
Carbon . .	82.68	82.49	82.84	83.02
Hydrogen .	12.04	11.90	11.77	11.95

The approximate yield of cholesteryl oleate did not vary greatly. Thus:

I.	500 c.c. serum yielded	0.24 gram	= 0.05 per cent
II.	610 c.c. "	0.19 "	= 0.03 "
III.	1660 c.c. "	0.78 "	= 0.05 "
IV.	2200 c.c. "	1.05 "	= 0.05 "

From over four litres of serum about 0.2 gram substance was obtained, having a melting point (after recrystallization) of 77–78° C. and thus corresponding with the cholesteryl palmitate. The crystalline form also confirmed this deduction.

Turkey serum. — In one instance a relatively large yield of cholesteryl oleate was obtained from turkey serum. Thus:

I.	600 c.c. serum yielded	0.37 gram	= 0.06 per cent
II.	1000 c.c. "	1.43 "	= 0.14 "

The preparations melted at 43–44° C. and one of them (II) showed the following composition on analysis:

Carbon	82.84 per cent
Hydrogen	11.79 "

A preparation corresponding in crystalline form and solubilities with cholesteryl palmitate melted (after recrystallization) at 77–78° C.

Goose serum. — From 220 c.c. of this serum about 150 mgr. (0.07 per cent) of characteristic rosettes of needles were obtained; they melted after recrystallization at 43–44° C., thus corresponding to cholesteryl oleate.

Duck serum. — Considerable difficulty was experienced in obtaining characteristic cholesteryl oleate preparations from duck serum. The products showed admixture of apparently amorphous material, and melted at about 60° C. From one half litre serum, however, about 0.4 gram (0.08 per cent) of crystals was obtained. After recrystallization, the crystals melted at 42–44° C. By extracting the serum residue with alcohol-ether, one half gram of a crystalline product was obtained. The preparation, when purified, melted at 76 °C., giving evidence of the probable presence of a considerable quantity of cholesteryl palmitate (melting, when pure, at 77° C.).

The blood-corpuscles. — That cholesterin may occur in an uncombined state in the blood-corpuscles seems probable from the recent analyses of Abderhalden,[1] who found noticeable quantities of cholesterin in them, while fatty acids were absent, or present only in very small amounts. The older statements of Hoppe-Seyler[1] lead to similar conclusions; and Wooldridge[2] stated that he obtained cholesterin free from fats and lecithin by extracting the stroma of blood-corpuscles with cold ether. In the present experiments crystals of cholesterin were repeatedly obtained from the blood-corpuscles by direct extraction with ether. The corpuscles were separated from defibrinated blood by centrifugalization or by subsidence, and after treatment once or twice with one per cent sodium chloride solution to remove any adherent serum, were rendered laky and extracted. The ether extract corresponded in its behavior with the description given by Wooldridge; on evaporation it yielded a residue of needle-shaped crystals occasionally arranged in rosettes. After recrystallization from alcohol-ether the more characteristic rhombic tables appeared. These crystals showed the characteristic color reaction with chloroform and concentrated sulphuric acid, and the absence of fats or fatty acids was demonstrated by the fact that they melted at a temperature above 100° C. The corpuscles of the sheep, dog, hen, and turkey were examined, and cholesterin crystals obtained in every instance.[3]

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[1] ABDERHALDEN: Zeitschrift f. physiol. Chemie, 1897, xxiii, p. 522; *Ibid.*, 1898, xxv, p. 108.

I desire to acknowledge the kind advice of Professor Lafayette B. Mendel, at whose suggestion these experiments were carried out.

---

[1] HOPPE-SEYLER and THIERFELDER: Handbuch der chemischen Analyse für Aerzte, p. 408.

[2] WOOLDRIDGE: Archiv für Physiologie, 1881, p. 389.

[3] Since the preceding account was sent for publication, a paper by E. Hepner: "Ueber den Cholestearingehalt der Blutkörperchen," has appeared in the Archiv f. d. ges. Physiol., 1899, lxxiii, p. 595. The cholesterin-content of the corpuscles of the dog and horse was determined; free cholesterin was also detected in the blood-plasma.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. II. — THE MECHANISM OF THE MOTOR REACTIONS OF PARAMECIUM.[1]

By HERBERT S. JENNINGS.

As pointed out in No. I. of these Studies,[2] most of the reactions of Paramecium (as well as other Protozoa) are known only in their general features. The mechanism of the reactions, — that is, the motions of the cilia by which they are brought about, has been determined for Paramecium only in Ludloff's study[3] of electrotaxis and my own of thigmotaxis. It is the purpose of the present paper to supply the lack thus indicated by giving a detailed account of the mechanism of the motor reactions of Paramecium.

The far-reaching importance of an accurate knowledge of the exact mechanism of the reactions of Paramecium is apparent when we recall the sweeping conclusions that have been drawn from the results of studies on the reactions of this and other unicellular organisms. Theories of the mechanism of thermotaxis, geotaxis, and other reactions have been put forth, accompanied by deductions as to the remarkable sensitiveness of protoplasm to the various classes of reagents, and these theories have borne an important part in forming various prevalent conceptions in general physiology and in comparative psychology. They depend for their validity on whether the mechanism assumed for the reactions is the correct one, and for this assumption a basis of observation has usually been lacking. As will appear, some conclusions that have been deduced with the utmost confidence lose their validity when the actual mechanism of the reactions becomes clear.

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[1] SCIENTIFIC RESULTS OF A BIOLOGICAL SURVEY OF THE GREAT LAKES, directed by Jacob Reighard, under the auspices of the U. S. Fish Commission, No. 1. (Published by permission of the Hon. George M. Bowers, Commissioner of Fisheries.)

[2] JENNINGS, H. S.: Journal of physiology, xxi, pp. 258-322.

[3] LUDLOFF: Archiv f. d. ges. Physiol., 1895, lix, p. 525.

It has become the fashion to refer many processes taking place in ontogeny to chemotaxis, or other reactions to stimuli, the matter apparently being considered as therewith definitely put at rest, as if such reference permitted no further analysis. It seems often to be forgotten that movements induced by chemotaxis must be accomplished by some means, some mechanism; and upon the character of this mechanism depends largely the interpretation to be given to the process. It may aid in recalling this neglected fact to set forth the roundabout method by which certain free cells succeed in gathering about a source of attractive stimulus.

A third standpoint, from which a knowledge of the general mechanism of reactions to stimuli should be of interest, is that of comparative psychology. The stage of mental development represented by unicellular organisms has, since the work of Verworn, been studied comparatively little, at least in any thorough manner, and the disconnected phenomena observed have lent themselves to interpretation by different authors in the most varied ways. Some hold that such organisms have a complex psychology containing nearly all the elements of which the psychic life of higher animals is made up, while others maintain that the observed phenomena are explicable on the simplest grounds; that such organisms are merely automata of the most limited capabilities, and that the activities which they show require little more than the property of irritability for their explanation. It may be hoped that an exact knowledge of the mechanism of the reactions of one of these organisms may throw further light on the question of the simplicity or complexity of the "psychic life of micro-organisms."

With these problems in mind, an examination will be made of the motor reactions of Paramecium. The organism to be studied, Paramecium caudatum,[1] is a unicellular animal belonging to the group of ciliate infusoria, and is well known in every biological laboratory, living by thousands in vegetable matter decaying in water. It is somewhat cigar-shaped in form, having a narrow but blunt anterior end and a broad, sharp posterior end. From the anterior end a broad depression known as the oral groove runs obliquely along one side (the oral side) to the mouth, in the middle of the body. Near the opposite side (the aboral side) are the two contractile vacuoles. The entire surface of the body is covered with cilia, by means of which the animal moves. The length of the animal is about 0.2 mm.

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[1] In the first of these Studies (*loc. cit.*) the organism used for experimentation is called Paramecium aurelia. I adopted this name because the organism has been always so called in the Jena Physiological Institute, where the work was done, and because my attention had not been called to the possibility of a mistake

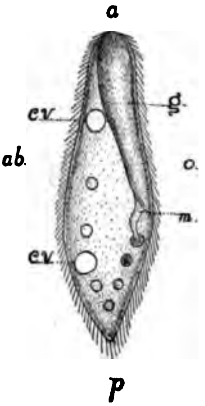

FIGURE 1. Paramecium caudatum. *a*, anterior end; *p*, posterior end; *ab*, aboral side; *o*, oral side; *m*, mouth; *g*, oral groove; *cv*, contractile vacuoles.

During its progress through the water, Paramecium comes in contact with sources of stimuli of various kinds; it strikes against mechanical obstructions, compelling it to turn aside, or it meets with various chemical substances in solution which either attract or repel it: heat, electricity, and other agents may likewise act as stimuli; to all these the animal reacts in a characteristic manner. The problem for solution is, exactly *how* is such a reaction accomplished? How does the attractive chemical compound succeed in affecting the locomotor organs of Paramecium so that it turns toward the source of stimulus? How does the repellent compound succeed in turning the Paramecium away from the source of stimulus?

In the first of these "Studies on Reactions to Stimuli" details were given as to the general features of the phenomena shown in the reactions of Paramecium. It was there shown that Paramecia are positively chemotactic to weak solutions of substances having an acid reaction, negatively chemotactic to substances having an alkaline reaction, and to many neutral salts. I shall give here in brief only enough of the gross appearance of these reactions to make intelligible the account of the finer mechanism and the way in which this mechanism is demonstrated.

in the name. But it appears that systematists make a distinction between Paramecium aurelia, with two micro-nuclei, and caudatum, with but one. As the Paramecia which I have been studying at Hanover, N. H., have but one micro-nucleus, I must call them P. caudatum. I believe them to be identical with the organisms used at Jena, though I did not investigate the micro-nucleus there.

The method by which the reactions of Paramecium toward most stimuli is demonstrated in practice is shown in Fig. 2. Paramecia are brought upon a glass slide and covered with a long cover-glass, supported near its two ends by bits of capillary glass tubing. The Paramecia swim at random throughout the preparation. Now a drop of any solution toward which the method of reaction is to be tested is introduced beneath the cover-glass by means of a capillary pipette, as shown in the figure. The Paramecia in their random swimming strike the edge of the drop, whereupon they react in a characteristic manner. If they are positively chemotactic to the substance in the drop — as they are if it is weakly acid in reaction — they soon gather in a dense swarm within the drop, as shown in Fig. 3. If, on the other hand, they are negatively chemotactic, the drop remains entirely empty, as shown in Fig. 4. This method is of course designed especially for the demonstration of chemotaxis. Thermotaxis, or the reaction toward heat or cold, may be demonstrated in a similar manner, or better by warming or cooling one end of the slide; still better by means of the apparatus devised by Mendelssohn.[1]

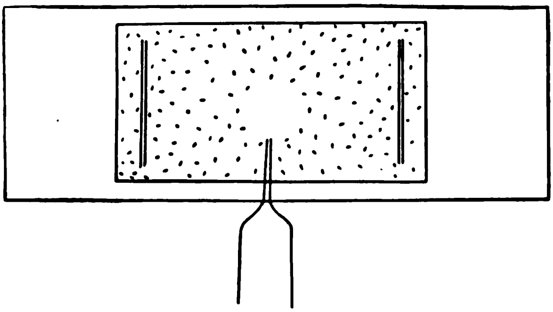

FIGURE 2. Method of testing the reactions of Paramecium.

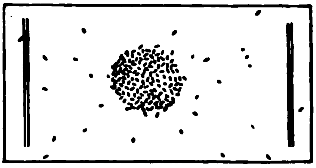

FIGURE 3. Paramecia collected in a dense swarm in a drop of a solution to which they are positively chemotactic.

Now, how does the substance in the drop in Fig. 3 succeed in affecting the cilia in such a way as to make the Paramecia turn toward and enter the drop?

Observation of the method by which the Paramecia collect in the drop shows that the foregoing question involves an assumption which is untrue. The Paramecia in the neighborhood of the drop do not turn toward it. The animals collect in the drop in an entirely different way. Suppose the drop to have been just introduced, as in Fig. 2. A Paramecium in its random course strikes by chance against the margin. It does not react, but keeps on its way across the drop until it comes to the other side (Fig. 5). There it reacts negatively, and turns back, swimming across the drop in another direction, till it again comes to the margin of the drop. There it again reacts negatively, and swims in a new direction, till it is turned back by the margin as before. This continues, so that the Paramecium is, as it were, imprisoned in the drop. Fig. 5 shows the course of a single Paramecium in such a drop. Other Paramecia enter the drop in the same way, purely by chance, and remain in the same manner, until the drop swarms with Paramecia. Owing to the restless hither and thither swimming of the animals on the slide, almost every one will in a short time have come by chance against the edge of the drop, will have entered and remained. Thus in a short time we get the appearance shown in Fig. 3; almost all the Paramecia in the preparation are collected in the drop. We say that the Paramecia are positively chemotactive to the substance in the drop, but so far as any motor reaction is concerned, evidently a more accurate statement is that after entering the drop by chance they are negatively chemotactic to the surrounding fluid.

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[1] MENDELSSOHN: Archiv f. d. ges. Physiol., 1895, lx, p. 1.

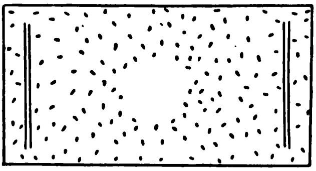

FIGURE 4. Negative chemotaxis toward a drop of sodic carbonate solution introduced 10 minutes before.

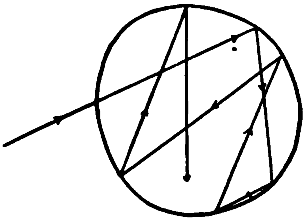

FIGURE 5. Course of a Paramecium in a drop of attractive substance.

The same thing may be shown to be true for thermotaxis or the reaction toward heat. In place of the chemical solution, we may introduce into a slide preparation of Paramecia in cold water a drop of water warmed to the temperature toward which the Paramecia appear to be positively thermotactic. We shall find that the Paramecia collect in the warm drop in exactly the same manner as in the case of the chemical compound just described: they enter by chance, then are negatively tactic to the surrounding cold water.

An extended examination of the reactions of Paramecium shows that this is typical for all apparently positive reactions. *In and of* *itself, considered as a motor reaction, there is no positive taxis in Paramecium.* The so-called positive taxis always acts indirectly through (apparent) negative taxis. The question of how Paramecium turns toward an attractive substance may be thrown out, with the simple statement that Paramecium does not so turn.

There remains then only the mechanism of the negative reactions to be elucidated. We may first proceed to an examination of the method by which Paramecium succeeds in keeping out of a drop of some chemical to which it is negatively chemotactic, as in Fig. 4. For this purpose a drop of $\frac{1}{8}$ per cent sodium chloride solution may be selected. To understand the negative reaction, it is necessary to recall the animal's normal method of progression through the water. The unstimulated Paramecium swims nearly straight forward, though in a slightly sinuous course, the narrower, blunter end directed forward. At the same time it revolves on its long axis. Usually, but not invariably, this revolution seems to be from left to right, but there is much variation in the direction.

Now, when a Paramecium, swimming as above described, strikes the margin of the drop of sodium chloride solution, it reverses its course, darting straight backward, — the broad, pointed (posterior) end now being in the lead. At the same time, the direction of rotation on the long axis is reversed, the Paramecium revolving (usually) from right to left. Next the animal swings on its short axis, so as to bring the longitudinal axis of the body out of the line of direction in which it was first swimming. Then the infusorian begins to swim forward again, — following a course which lies at an angle to the course it was taking when it struck the drop of sodium chloride solution. Briefly stated, the animal has adopted the very rational course of backing off, turning in a different direction, then proceeding on past the obstacle, — much as any higher animal would have done.

We may here take up two questions, upon one of which we have already a certain amount of evidence, while the second is pressed upon us by the course of events in the reaction last described. The first is, — Do different kinds of stimuli have different methods of affecting the cilia? For example, does perhaps an acid cause the cilia to strike more strongly backward, driving the animal forward; an alkali, on the contrary, cause the cilia to strike more strongly forward, driving the animal backward? In this way, at first thought, the positive chemotaxis toward acids, the negative taxis toward alkalies might be explained. The results detailed thus far have shown, however, that such a view is not tenable for an explanation of positive chemotaxis, since the latter acts only indirectly through negative chemotaxis. Nevertheless, the general question still remains, — applicable, if not to acids and alkalies, perhaps to other chemical or physical agents. To determine the question with certainty, it will be necessary to immerse the Paramecia directly into solutions of various sorts, and observe the activities of the animals under these circumstances. At the same time evidence may perhaps be obtained on the second question referred to above, which is as follows: What causes the *turning away* of the Paramecium in the reaction toward sodium chloride, above described, after the backward course; and what determines the direction in which it turns? An obvious answer of course suggests itself, — the Paramecium turns away because it wants to get around the obstacle, and the direction in which it turns is determined by the position in which the object lies; it turns so as best to avoid the obstacle. This anthropomorphic view may perhaps be tested, and some evidence on our general question gained, by trying the effect of unlocalized stimuli, — stimuli that act upon the entire surface of the animal at once. This may of course be done by the method above mentioned, — complete immersion of the Paramecia in solutions of different kinds. There will then be no "obstacle" to get by, and, from the anthropomorphic standpoint, no excuse for turning in one direction or another.

With these considerations in mind, Paramecia are immersed directly in solutions of various sorts. This is done as follows: a solution is mixed in a watch-glass, then a quantity of water containing Paramecia is taken up with the pipette and injected energetically into the solution. They are quickly mixed thoroughly by the use of the pipette, then examined with the low power of the microscope.

The Paramecia may be first immersed in an alkaline solution, using for this purpose $\frac{1}{200}$ per cent sodium hydrate. Most of the Paramecia will be seen to swim energetically backward. This may be continued a few seconds or more, then many of the animals will be seen to turn and swim forward in a new direction. If a single specimen is watched, it may perhaps be seen to repeat the process of swimming backward, turning, and swimming forward several times; others may swim backward continuously.

Thus it appears that an alkali causes a reversal of the motion, followed by turning and swimming forward.

Let the Paramecia now be immersed in $\frac{1}{50}$ per cent acetic acid. As in the sodium hydrate, the Paramecia dart backward, only here the motion is more swift. The action of the acid seems much more energetic than that of the alkali; the Paramecia dart furiously backward for an instant, then turn quickly and rush forward for a moment, then perhaps backward again, then turn and rush forward. In a short time the backward motion has ceased; the Paramecia are all swimming forward at a furious rate, and soon they die. The difference in the action of the substances seems due to the fact that acids do not affect Paramecia at all, except when they are so strong as to be severely injurious; hence the great energy of the reaction and the quick death of the animals.

Thus acids also cause the infusoria to swim backward at first. The reaction is qualitatively the same for both acids and alkalies, only the intensity and duration of the different parts of the reaction varying.

In the same way, Paramecia may be immersed successively in a solution of some neutral salt, as $\frac{1}{5}$ per cent sodium chloride; in a solution that is effective only through its strong osmotic power, as a 10 per cent solution of cane sugar; in a watch-glass of water heated to 35° C.; in a watch-glass of water at the freezing temperature. In every case the characteristic features of the reaction are the same. The Paramecia swim backward for a longer or shorter time, then turn more or less, then swim forward. This may be repeated many times. In the different solutions the different features of the reaction may be more or less pronounced, in energy and duration, but the essential nature of the reaction is the same in all.

This series of experiments gives a definite answer to the first of the two questions proposed. Evidently different stimuli do *not* have qualitatively different methods of affecting the cilia. In every case the essential features of the reaction are the same, whatever the agent causing the reaction; only the intensity of the component activities varies. Every reagent causes at first a reversal of the motion of the animal, and therefore beyond doubt a reversal of the motion of the cilia. Since the same result is obtained with stimuli of so heterogeneous and opposite a character, it follows that the determining feature in this method of reaction must be internal. The Paramecium is like a machine in which the wheels are set for a certain motion; to every application of power from whatever source it responds by this motion. It is to be noted, moreover, that while in these experiments the stimulus is continuous, the reaction is discontinuous, the motion being first backward, then sideways, then forward. The demonstration that a single stimulus does not have a single characteristic effect is therefore complete, since each stimulus causes three different motions of heterogeneous and even opposite character.

On the second question, as to what causes the turning away, and what determines the direction of turning, these experiments shed a certain amount of light, though they do not give a determinate answer. Since all these entirely unlocalized stimuli cause the turning away, it follows that the latter is not due to the localization of the stimulus: the Paramecia do not turn from nor toward the source of stimulus, — because they are completely surrounded by and immersed within it. Apparently the turning away is due, like the reversal, to an internal mechanism, not to any characteristic of the external stimulus. Neither can the *direction* in which they turn be determined by an external factor in these cases. It appears probable, therefore, that it is an internal factor that determines the direction of turning.

The next step is to try whether anything can be discerned in the structure of the Paramecium that will give a clue to the factor determining the direction of turning. The most obvious differentiations of the body of the animal are into anterior and posterior ends, and into oral and aboral sides, as shown in Fig. 1. The differentiation of the ends comes into consideration, of course, only in forward or backward motion. Has the differentiation into oral and aboral sides any relation to the turning movement? Owing to the continued rotation of the animal on its long axis, as it swims either backward or forward, it is impossible under ordinary conditions to observe whether there is any relation between this differentiation and the direction of turning. But a clue is given by the conduct of the animals when immersed in a solution of potassium iodide. This reagent has the property of almost indefinitely prolonging each of the component activities in the threefold reaction above described, and affords opportunity for a thorough analysis. When introduced directly into 1 per cent solution of potassium iodide the Paramecia begin, as in other reagents, to swim backward. But in potassium iodide solution this is continued six to eight minutes without change. After this they begin to turn, and this portion of the reaction is continued like the first, so that they spin around on the short axis of the body, as on a pivot, for half an hour at a time. Thus after ten minutes all the Paramecia in the watch-glass will be whirling like tops. It is easy thus to have hundreds of specimens in the field at once with a low power lens, and of these many individuals will be in such a position that it is easy to determine the relation of the direction of turning to the two sides of the animal. It then appears that all are whirling toward the side that contains the contractile vacuoles, — that is, ***toward the aboral side.*** The direction of motion is indicated in Fig. 6. At first the Paramecia whirl as if on a pivot through the middle of the animal. Later the centre of rotation comes to lie outside the body of the animals, so that they now swim in circles. In every case the centre of the circle lies on the aboral side of the Paramecium, so that the animal is still turning toward the aboral side, though not in such short turns as at first.

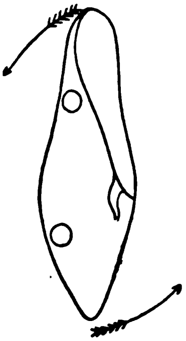

FIGURE 6. Direction of motion of Paramecia whirling in 1 per cent solution of potassium iodide.

Thus under the stimulus caused by potassium iodide, the direction of turning is determined by an internal factor, — the differentiation into oral and aboral sides being the deciding feature. Taking this in connection with the fact that all unlocalized stimuli cause turning, it is a natural inference that the localization of a stimulus has no effect on the direction of turning. As this seems paradoxical and improbable on general principles, however, it must be tested by observation of the reactions of Paramecium toward stimuli that are localized. Is there a difference in the reactions of the animal according as the stimulus affects the anterior end, the oral side, the aboral side, or the posterior end? To determine the answer to this question, it is only necessary to introduce a drop of some chemical compound beneath the cover-glass of a slide preparation swarming with Paramecia, as shown in Fig. 2, and then to watch the edge of the drop with the low power of the microscope. Among the multitudes of Paramecia that strike the margin, many come against it obliquely, or touch it only on one side of the body, and their conduct is then easily observed. The reaction is the same in character as when the animal is immersed in a solution that acts as a stimulus; the Paramecium swims backward a distance, turns, and swims forward. It becomes evident at once that it *does not* turn directly away from the source of stimulus; the position of the drop seems to have little or no relation to the direction in which the Paramecium turns. Sometimes the animal turns in such a way as to carry it away from the drop; in other cases it does not. There is no constancy in the direction of turning, and it is impossible to predict which way a Paramecium meeting the drop at a given angle will turn. In many cases the animal which at first brushed only the edge of the drop is carried, after turning, squarely against it, as illustrated in Fig. 7, in which the numerals indicate successive positions of the animal, while the arrows show the direction of motion. In such a case the whole operation is repeated; the animal backs off, turns in a new direction, and tries again. If the obstruction is large, the Paramecium may be thus compelled to try a dozen times before getting by the obstacle; such cases are often observed.

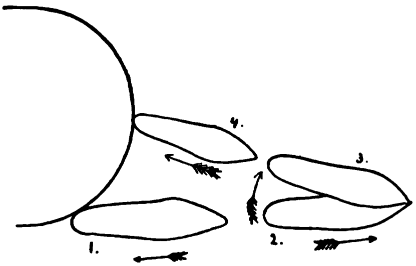

FIGURE 7. Paramecium striking a drop on one edge (1); swimming backward (2); turning (3); and swimming forward squarely against it (4).

This description applies equally well to the case of a mechanical obstruction, and to the reactions toward a very hot or very cold solution.

The inference previously made, that the localization of the stimulus has no relation to the direction in which the Paramecium turns, is therefore confirmed. It remains now to be decided whether the factor which determines the direction of turning when the stimulus is a solution of potassium iodide is the factor in other cases also. Does Paramecium always turn toward its aboral side, whatever be the nature and position of the stimulus?

To answer this question, it is necessary to use the method of observation in gelatine, devised by Jensen. Three grams of gelatine are dissolved by heat in 100 c.c. of ordinary water, and the Paramecia are observed in the thick solution. In this gelatine solution they are able to swim only very slowly, so that all the movements can be closely studied. Any chemical can be mixed with the gelatine solution, in order to observe the effects of unlocalized stimulation, or a drop of any solution can be introduced beneath the cover-glass with the capillary pipette, for observation of the effects of localized stimuli; in fact, the Paramecia may be studied exactly as in water, the only essential difference being that they move more slowly.

The Paramecia are therefore brought into such a gelatine solution, and their conduct toward localized stimuli is observed. The unstimulated Paramecium swims straight forward, revolving slowly on the long axis. On coming in contact with a mechanical stimulus or a drop of some solution toward which it is negatively chemotactic, the animal swims backward very slowly, at the same time revolving slowly from right to left. Then it turns slowly, — *always toward the aboral side*. If the aboral side is above when the turning begins, the animal turns upward; if the aboral side is below, it turns downward; if to the right the animal turns to the right. This is true for a simple mechanical stimulus, for a chemical stimulus of any sort, or for a thermal stimulus; in fact, for any kind of a stimulus that can be tested in the gelatine. After some time in the gelatine solution the Paramecia are usually seen to be somewhat curved, and the curving is always toward the aboral side, as in Fig. 8. A similar curving may often be observed after a strong stimulus, when the Paramecia are mounted in water. The anterior end thus curved of course acts like the rudder of a boat in turning the course of the Paramecium toward the aboral side. Nevertheless, while it aids, it is evident that this is not the only factor in the turning, since Paramecia under other conditions are seen to turn sharply, or even to revolve almost as if on a pivot, when the body is not observably curved at all.

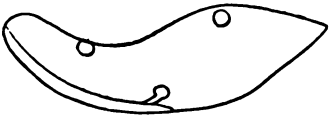

FIGURE 8. Paramecium curved toward the aboral side, under the influence of a powerful stimulus.

The invariable turning toward the aboral side may, as I have since discovered, be demonstrated, without the use of gelatine, in the following way: Paramecia intermingled with masses of bacterial zoöglœa are mounted on a slide and covered with a cover-glass, which is closely pressed down, so as to leave only a narrow space between slide and cover. It will usually be found that many Paramecia are imprisoned in small crevices in the bacterial zoöglœa, so that they can swim only from one end of the crevice to the other, and at each end they must turn. The cover-glass is so close to the slide that the animals can turn only in a horizontal plane. By watching the Paramecia as they turn at the end of the crevice, it will easily be seen that they invariably turn toward the aboral side.

We find then that the question last proposed is to be answered affirmatively. Paramecium after reversing *always turns toward the aboral side*, whatever be the nature and position of the source of stimulus.

A certain feature in the normal straightforward swimming of the unstimulated Paramecium appears to be connected with this fact. As has been often noted, the course in which an individual swims is not perfectly straight, but, as seen from above, the animal seems to swerve from side to side. This swerving has been figured by Verworn[1] and others. The actual path is a narrow spiral, the animal, of course, swerving up and down as well as from side to side. Now, observation of slowly moving individuals shows that at any point in this spiral course the aboral side is on the outside of the spiral. In other words, the Paramecium as it swims continually swerves toward the aboral side. As at the same time it rotates on its long axis, the position of the aboral side continually changes, so that the resulting path is a spiral one, and the general result is the same as if the Paramecium had progressed in a straight line. These facts are indicated in the accompanying diagram of the path of a Paramecium (Fig. 9). Apparently the method of reacting to a stimulus by turning toward the aboral side is a mere accentuation of the ordinary swerving toward that side.

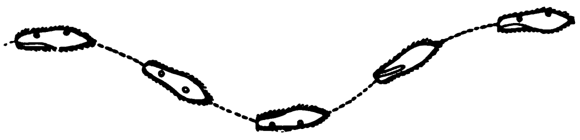

FIGURE 9. Path of a Paramecium. The aboral side is everywhere directed toward the outside of the spiral.

It will here be necessary, in order to avoid misunderstanding, to give an account of an apparent exception to the rule that the Paramecium after stimulation always turns toward the aboral side. In my previous paper on the reactions of Paramecium (*loc. cit.*), I gave an account of the thigmotactic reaction of Paramecium; that is, the reaction due to continued contact with a solid body. I must here supplement the account by setting forth a little more fully certain features in that reaction.

On page 303 of my previous paper is figured a Paramecium resting with its anterior end against a solid body, and exhibiting the currents caused by the cilia under these circumstances, — that is, the currents characteristic of the thigmotactic reaction. As described in detail in the paper cited, the cilia in the oral groove at such a time beat strongly backward, driving a swift current of water toward the rear and causing a vortex to form on the oral side of the anterior half of the animal, as shown in Fig. 19 (*loc. cit.*), while the cilia on the remainder of the body are quiet, or possibly have varying motions tending to counteract the motion of translation that might be induced by the cilia in the oral groove. Meanwhile, the body of the animal remains at rest. This type of activity of the cilia is especially characteristic for resting Paramecia. But sometimes the solid body which causes the thigmotactic reaction is very small and movable, and so does not restrain in any way such motions of the animal as might naturally result from the activity of the oral cilia. In this case the Paramecium moves through the water in a peculiar way, carrying with it the small particle that has induced the thigmotactic reaction. "The cilia beating strongly backward in the oral groove of course impel the animal forward. But as the active cilia are all on one side, there is also a tendency to move toward the opposite side. The resultant of these two motions at right angles to each other is a motion in the circumference of a circle. In fact, the animal moves forward in exactly the lines indicated by the arrows in Fig. 19, only, of course, in the opposite direction from the water currents. The ciliary motion is thus the same whether the thigmotactic animal is at rest or in motion; in the former case the water currents move obliquely backward; in the latter case the animal moves on the same lines obliquely forward. The Paramecium is, as it were, whirled about in its own whirlpool" (*loc. cit.*; p. 305). An inspection of the Fig. 19 referred to in this extract shows that when the animal moves in a circle as there described, the oral side of the Paramecium must look continually toward the centre of the circle. Fig. 10 shows the successive positions of a Paramecium exhibiting the thigmotactic reaction toward such a movable particle; the arrows indicate the direction of motion of the Paramecium, while the circle indicates the path traced by the anterior end of the animal. By reference to this figure it will be noticed that under these circumstances the body moves forward tracing a curve, with the oral side always toward the inside of the curve; in other words, the animal continually turns *toward the oral side.* In connection with the other movements de-

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[1] VERWORN, M.: Psychophysiologische Protisten-Studien, 1889, p. 116.

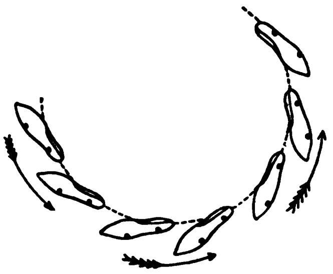

FIGURE 10. Motion of Paramecium in the thigmotactic reaction to a movable particle.

scribed in these pages, it is important to recall the fact that Paramecia will be sometimes seen thus turning toward the oral side; this, however, is always in the thigmotactic reaction. It is especially frequent when Paramecia are observed undisturbed in water containing many bacteria; when the Paramecia are excited by some unusual condition this reaction is not seen. Now, it is chiefly the reaction under some exciting stimulus that forms the subject of this paper. The thigmotactic reaction is characteristic for resting individuals, and the locomotion in thigmotaxis under certain circumstances in the manner shown in Fig. 10 is an accidental matter, due to the mobility of the solid inducing the reaction. Hence, this is not properly to be accounted a motor reaction, and it is, therefore, with this brief description, left out of consideration in my discussion.

Returning, therefore, to the proper motor reactions, the final step must now be taken; the mechanism of the reactions already described must be determined in terms of the movements of the cilia. All movements of the Paramecia result from motions of the cilia, and the mechanism of the reactions cannot be considered as analyzed into its simplest components until we know what motions of the cilia are at the basis of the general body movements. For studying the motions of the cilia two methods may be used. The first is the observation of the animals in the gelatine solution in the way already given. The thickness of the solution makes the motion of the cilia much less rapid, so that the way in which they move can be determined more or less satisfactorily. The data gained in this way may be supplemented and controlled by the second method of study. This consists in observing the Paramecia in fluid containing powdered carmine. The movements of the particles of carmine in the neighborhood of the animals show the currents caused by the cilia, and from the direction of these currents the direction of stroke of the cilia causing them may be inferred.

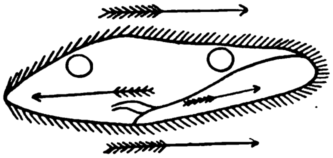

FIGURE 11. Paramecium with cilia reversed. The large arrow in the body shows the direction of locomotion of the animal; the other arrows indicate the direction of the currents of water.

In the unstimulated Paramecium swimming forward the effective stroke of all the cilia is backward, urging the animal forward; the cilia as seen in gelatine give the optical effect of being directed backward, as shown in Fig. 1. On coming in contact with an effective source of stimulus, the cilia are reversed in position, now striking more strongly forward, and presenting the appearance of being directed toward the anterior end, as in Fig. 11. As a result the animal is, of course, impelled backward, or toward the broad, pointed end. It is worthy of especial notice that the cilia in the oral groove are, like all the others, now striking forward, so that a current of water passes swiftly *from* the mouth toward the anterior end, — contrary to the usual direction.

Next, after the backward swimming caused by the reversal of the ciliary motion has ceased, the animal begins to turn. At this stage the cilia are found to have the following motions. The reversal of the cilia in the oral groove has ceased, and they are striking backward again, — thus tending to drive the animal forward. The remainder of the cilia on the anterior half of the animal strike *transversely toward the oral side.* This is shown in Fig. 12. This reaction was first seen clearly in Paramecia that were immersed in a $\frac{2}{5}$ per cent solution of chrome alum; it was then confirmed for other substances and for stimuli other than chemical, by the use of the gelatine solution and by observation of the currents about Paramecia in water containing powdered carmine. The arrows in Fig. 12 indicate the direction of the water currents produced by the cilia at this stage of the reaction. In the oral groove there is a strong current backward, while on the anterior half of the aboral side there is a transverse current from the aboral to the oral side. This motion of the cilia of course causes the Paramecium to turn toward the aboral side (opposite the arrows in Fig. 12), and with this observation we have determined the real mechanism of the turning.

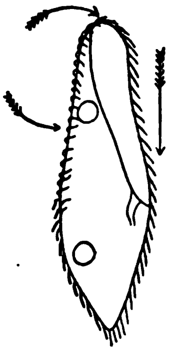

FIGURE 12. Position of the cilia as the Paramecium turns. The arrows indicate the direction of the water currents.

The next step in the reaction is the cessation of the transverse stroke of the aboral cilia, so that they strike backward, as before the stimulation, driving the animal straight forward as at first.

Thus the reaction of Paramecium to any ordinary stimulus is as follows: (1) The cilia over the entire surface of the body are reversed, striking forward, thus driving the animal backward. At the same time its direction of revolution on the long axis is reversed. (2) The reversal of the cilia ceases; those in the oral groove strike backward as at first, while those on the remainder of the anterior half of the body strike transversely, toward the oral side. This results in turning the anterior end toward the aboral side. (3) The cilia all strike backward as at first; the animal, therefore, swims straight forward again. Coincidently with (1) and (2) the body of the animal may be bent toward the aboral side, still further aiding in turning in that direction. At the end of the entire manœuvre the Paramecium is swimming forward along a course which lies at an angle to the course in which it was first swimming. The complete reaction of Paramecium to an ordinary stimulus is represented in Fig. 13. The rotation on the long axis could not be represented in this diagram, but all the other essential features of the reaction are shown.

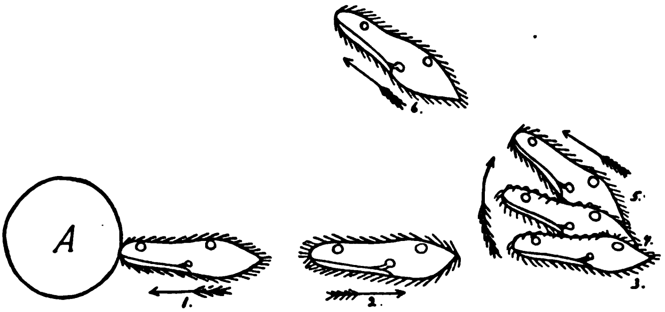

FIGURE 13. Complete reaction of a Paramecium. *A* is a source of stimulus. The numerals give the successive positions of the animal, while the arrows show the direction of motion.

This reaction is not characteristic for any particular class of stimuli, but it occurs in a more or less marked manner whenever any stimulus acts upon the animal with sufficient power to cause a motor reaction. Toward acids and alkalies, toward neutral salts and toward fluids that are active only through their high osmotic pressure; toward heat and cold and mechanical shock, the same reaction is given. Apparently a strong electric current has a related effect, since Verworn has observed that when a very strong electric current is passed through water containing Paramecia, they all swim backward, and thus collect at the anode instead of at the cathode, as is usual with a weaker current. A re-examination of the phenomena of electrotaxis in the light of the general reaction-plan described in this paper appears desirable to determine the relation of the two. Geotaxis, or the reaction of Paramecium relative to the direction of the pull of gravity, in virtue of which they usually rise to the top of a vessel of water containing them, seems more difficult to bring into relation with the general reaction above described. In view of the divergent theories of Jensen,[1] Davenport,[2] and others, the cause of geotaxis cannot be considered certainly known; a reinvestigation of the phenomenon in Paramecium is needed, in the light of the general method of reaction described in this paper, as well as in view of the previously unsuspected positive chemotaxis toward carbon dioxide (as described in No. I of these Studies) — a substance the distribution of which in the water profoundly influences the movements of the Paramecia. Like Miss Platt,[3] I find that the Paramecia of this region do not show clear geotaxis.

Almost the only variation in this entire reaction is in the intensity of its several component activities. To a very weak stimulus, such as a mechanical obstruction in front, the Paramecium responds merely by a reversal of the cilia lasting but an instant and carrying the Paramecium backward but a fraction of its own length. The accompanying reversal of the direction of rotation on the long axis may suffice to turn the aboral side but a few degrees from its original position. The reversal is followed by a momentary transverse stroke of the aboral cilia, turning the Paramecium through but a small angle toward the aboral side. It then swims forward as before. The whole operation has caused a hardly perceptible stoppage in the course of the Paramecium. On the other hand, coming in contact with some strong chemical, such as a drop of $\frac{1}{100}$ per cent sulphuric acid, induces an intense and long sustained reaction. The Paramecium darts swiftly backward many times its own length, revolving rapidly on its long axis from right to left. Finally, the transverse stroke of the aboral cilia intervenes, in an equally powerful manner, and the animal turns sharply. It may thus turn completely around; or in some cases it may whirl more than 180°, so that the immediately following forward course takes it back again into the drop of acid. Certain chemicals exaggerate all the activities composing the reaction to a remarkable degree. Thus in $\frac{3}{4}$ per cent potassium iodide, as already described, the reversal of the cilia lasts six to eight minutes, the Paramecia all this time swimming swiftly backward; then the transverse stroke of the aboral cilia lasts perhaps half an hour, the animal all this time whirling toward the aboral side. In such cases the reaction loses all semblance of an attempt to escape or pass by an obstruction. In other chemicals each component part of the reaction is little exaggerated, but the entire operation is repeated many times, alternations of swimming backward, turning, and swimming forward again continuing for fifteen minutes or more. Different chemicals have a most varied effect on the reaction as a whole, though in every case the effect may be expressed as a modification of the intensity or duration of one or more of the component activities.

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[1] JENSEN, P.: Archiv f. d. ges. Physiol., 1893, liii, p. 428.

[2] DAVENPORT, C. B.: Experimental morphology, Part I.

[3] PLATT, JULIA B.: American naturalist, 1899, xxxiii, p. 31.

The absolute direction in which the Paramecium turns after its backward course is evidently determined by the position of the aboral side when the cilia on that side begin to strike transversely. The position of the aboral side at that time is a matter of chance; the animal revolves continually on its long axis, so that the position of the aboral side is continually changing, and the actual direction in which the Paramecium turns depends upon where the aboral side happens to lie when the reaction reaches the stage in which the aboral cilia begin to strike transversely. Of course this may carry the animal directly into the source of stimulus again; such cases are often observed, as has already been described and illustrated in Fig. 7. In many cases the Paramecium repeats the reaction several or many times before it succeeds in getting by the obstacle. The whole proceeding is an apotheosis of chance. If the source of stimulus is small, the process of drawing back and turning in any chance direction is likely to avoid it. Or, if it is not avoided the first time, the continual revolution on the long axis is likely to bring the aboral side into a new position next time, so that the Paramecium turns in a new direction. If this is continued long enough, a small obstruction is certain, by the laws of chance, to be evaded and passed after a sufficient number of trials. If, on the other hand, the source of stimulus is large; if, for example, the space containing the Paramecium is bounded along one whole side by a solution toward which the Paramecium is negatively chemotactic, then the reaction is repeated until by the laws of chance the course of the Paramecium is so directed as to lie in that part of the space which does not contain the repellent solution. In the case of a solution toward which the Paramecium is strongly negatively chemotactic, chance may be aided by the fact that the intensity of the stimulus usually causes an intense reaction, so that the Paramecia swim a considerable distance backward (which in itself, of course, increases the chance of avoiding an obstacle after turning), and also turn strongly, — perhaps nearly 180°, so that the remainder of the path lies in quite a new direction. It seems probable that for most circumstances in the normal daily life of a Paramecium, this rough hit-or-miss method of reaction is quite sufficient to keep the animal out of danger and to enable it to avoid obstacles. But the utility of the reaction is left to chance, and so circumstances may arise in which the chances are against the Paramecium. Thus in the presence of a drop of some strong chemical, such as $\frac{1}{100}$ per cent sulphuric acid or cupric chloride, it will readily be seen that the chances of escape are limited. The animal responds to the first stimulus by a violent reversal of the cilia, carrying it some distance backward. If now the direction in which the transverse stroke of the aboral cilia carries it after this backward course is by chance such as to take the animal away from the drop, well and good; if, on the other hand, the Paramecium is carried a second time against the drop, it is likely to succumb at once to its poisonous effects. Observation shows that when a drop of such a substance is introduced into a preparation of Paramecia on the slide, it soon becomes surrounded by a zone of the dead infusoria. The same is true of a drop of a dense solution of cane sugar, which is injurious from its osmotic pressure. The Paramecia react when they enter such a dense solution, but the reaction is not precise enough to take them out of the destructive area before they are killed.[1]

The uselessness of the reaction seems especially striking when the Paramecia are completely immersed in a solution that acts as a stimulus. Under these circumstances, when there is no obstacle to be avoided and the stimulus is not localized at all, the Paramecia respond with exactly the same reaction. Paramecia do not modify their conduct to suit the needs of the case, but give the same reaction under all circumstances. The reaction is exactly such a one as might be maintained by natural selection, acting under an environment that makes no severe demands. Since the Paramecium usually swims forward, a majority of the stimuli that affect it at all will affect the anterior end. The whole reaction to *any* stimulus is, therefore, based upon this position of the source of stimulus, without regard to the conditions in a given particular case. For stimuli acting upon the side of the body, the reaction is, therefore, a very imperfect one, the comparative rarity of such stimuli having led to no development of a special mechanism to suit that case. A reaction such as would permit a direct turning away from a stimulus acting upon any side of the animal would evidently involve a much more complicated mechanism, and imply a higher condition psychologically than this simple one-reaction plan. But even this serves, if repeated a sufficient number of times, for avoiding sources of stimuli acting upon the sides of the animal, and under some conditions the result is produced in a fairly direct manner. In certain cases, when the stimulus causing the reaction is very weak, and the Paramecium comes against the source of stimulus very obliquely, so as to touch it only on one side, there appears to be an almost direct turning away, toward the side opposite the stimulus. This is to be observed particularly in the collections of Paramecia in a region containing carbon dioxide excreted by themselves. A long narrow area may thus be formed beneath the cover-glass (as shown in Fig. 14), in which the Paramecia swim back and forth; to the water outside this area they are weakly negatively chemotactic, so that when they swim against the boundaries of the area, the above-described reaction takes place, in a not very pronounced manner. A single Paramecium swimming lengthwise of this area may strike against its lateral boundaries with one side or the other, when it will frequently (not always) be seen to apparently sheer directly off, away from the boundary, and keep on its course undisturbed. Careful observation shows that such cases are to be placed in two categories. (1) When the Paramecium comes against the boundary, the aboral side by chance already lies toward the interior of the area, so that all that is necessary is for the Paramecium to react in the usual manner, in order to leave the boundary. (2) The more interesting case is where the Paramecium comes against the boundary with the aboral side *not* toward the interior of the area. In this case it will be seen to hesitate an appreciable interval before turning away from the boundary. During this moment of hesitation it *starts* to turn toward the aboral side, which is thereby pushed further into the outer fluid, toward which the animal is negative. This induces a faint reversal of the cilia (in agreement with the general

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[1] There is great variation in the repellent power of different chemical compounds. This subject will be treated by the author in a separate paper.

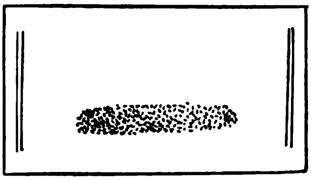

FIGURE 14. Group of Paramecia in a region containing carbon dioxide excreted by themselves.

plan of reaction above described), so that the animal does not progress further in that direction, and may be seen to move backward a barely perceptible distance. It then tries the turning again. On account of the continual rotation on the long axis, the aboral side will now be in a new position; the animal will, therefore, turn in a new direction, and may swim victoriously into the area; if not, a new trial is made during another instant of seeming hesitation. The delay cannot be longer than it requires for the Paramecium to rotate half a turn on its long axis. The animal makes the appearance of feeling about with its anterior end, to discover the proper course to pursue; it is really trying all the time to turn toward the aboral side, but cannot go forward until this carries it into a medium that does not cause a reversal of the cilia. The direction in which the animal shall go is not selected by the Paramecium itself, but is *automatically* selected through the fact that this direction does not cause a reversal of the cilia.

This more direct method is, however, only possible in cases where the negative stimulus is very weak, so as to cause only a very faint reversal of the cilia. With a stronger stimulus the animal shoots back as much as half its length, or more, so that it is now separated from the source of stimulus by a perceptible interval (as in Fig. 13); it is, therefore, free to turn in any direction. Hence, it as frequently turns at first *toward* the source of stimulus as in any other direction. Moreover, even with the weakest stimulus, not all individuals turn in the more direct way just described; many individuals in such an area as shown in Fig. 14 react essentially as shown in Fig. 13. The latter figure shows the *typical* reaction, of which the method just described is a less usual special case.

For the case of complete immersion in a solution that acts as a stimulus, the reaction seems under experimental conditions to be ludicrously imperfect and useless. Under the normal conditions, however, it perhaps serves the Paramecium very well even for this case. Suppose that the Paramecium in its headlong course finds that it has entered completely a circumscribed region of solution having injurious properties. Evidently, to immediately reverse the cilia and swim a long distance straight backward is as likely to rescue the Paramecium from its perilous position as anything it could do, and if this straight course backward does not bring it at once out of the injurious solution, it is perhaps likewise sound practice to change the direction of motion. As under other circumstances, the reaction depends upon chance for success, but the chances are fairly favorable. It is interesting to see how so simple and invariable a method of reacting can meet so well the demands made upon it.

Finally, for the very rare case of stimuli acting upon the posterior end, no provision whatever seems made. It is only when swimming backward in response to a stimulus from in front that a stimulus is likely to act upon the posterior end, — and this is apparently so rare that it can safely be neglected. In cases where animals swimming backward come in contact with something that would act as a stimulus if it affected another part of the body, the cilia continue to strike forward. The normal reaction would be of course to reverse the stroke of the cilia, making them strike forward; as, however, they are already striking forward, there is now no change. Thus Paramecia swimming backward may often be seen to press the posterior end against a mechanical obstruction met in the backward course, and remain there, the forward stroke of the cilia driving them continually against it. Or in the case of chemical stimuli, Paramecia swimming backward often cross the boundary between two substances, when this boundary seems an insuperable obstruction to individuals swimming forward. This is particularly striking when a drop of weak acid is introduced into a slide of Paramecia. As detailed in my previous paper (*loc. cit.* p. 269, Figs. 7 and 8), the Paramecia gather in a ring about such a drop, and are then negatively chemotactic to the inner, more strongly acid part of the drop, and also to the outer fluid. Therefore, they swim about in a ring of some width, surrounding the drop of acid; if they strike against either the inner or the outer boundary of this ring, the cilia are reversed, so that the animals return into the ring. But if a Paramecium on striking the drop of acid at the inner boundary of the ring reverses its cilia strongly and swims backward, it may, if it reaches the outer boundary while swimming backward, cross this boundary without the slightest hesitation, and swim away into the surrounding water, though if it had come against the outer boundary while swimming forward, it would have been at once forced back into the ring by the reversal of its cilia. In cases, however, where a Paramecium swimming backward comes in contact with a source of very strong stimulus, the next step in the reaction is induced, — the Paramecium begins whirling on its short axis, — a proceeding which serves a purpose as little as would the continued swimming backward.

The collection of Paramecia in the region of a given attractive stimulus, as in positive chemotaxis or positive thermotaxis, evidently depends equally upon chance. Nothing acts as a direct attraction to Paramecia. Their habit of swimming continually through the water is what must be relied upon to bring them into the sphere of activity of any agency. In this seemingly random swimming they are frequently met by influences which cause in a slight degree the reaction I have described, as is shown by the frequent slight jerks backward, followed by a slight turn in a new direction. In this way the Paramecium follows the line of least resistance, as it were, and finally may come into the region of a chemical toward which it is positively chemotactic, or into a region of optimum temperature. If now it proceeds on its course across the optimum territory, on coming to the farther boundary it passes into a region of such a nature as induces the negative reaction; the animal turns away and after some repetitions is directed back into the optimum region. The actual process by which this takes place in the case of thermotaxis may be detailed, since my account differs so completely in principle from that of Mendelssohn,[1] who first gave a careful description of thermotaxis, — and since this process affords a very striking example of what might be called the automatic selection of movements.

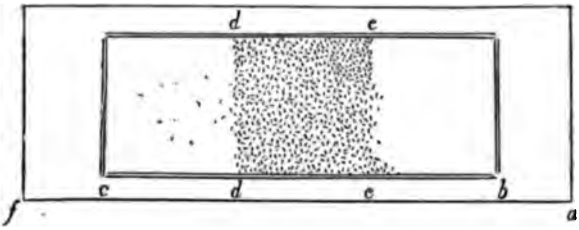

FIGURE 15. Thermotaxis of Paramecium. The right end from *a* to *c* rests upon a water bath heated to 40° C.; the opposite end, from *f* to *d*, rests upon ice.

Four strips of capillary glass tubing, enclosing a rectangular space, are fixed on a slide by means of Canada balsam (Fig. 15); the enclosure is then filled with water containing Paramecia, and covered with a long cover-glass. The Paramecia are scattered uniformly throughout the enclosure, swimming in all directions (the two ends being at first not empty, as they appear in the figure). Now one end of the slide for about a fourth of its length (from *a* to *c*) is allowed to rest on a water bath heated to about 40° C., while the other end, from *f* to *d*, rests on a piece of ice. Soon it will be noticed that the Paramecia at the heated end, between *b* and *c*, are in violent motion. They swim rapidly, some forward, some backward, now and again turning quickly and shooting in a new direction. No particular direction is preferred; the animals simply swim swiftly in any chance direction. Now those which by chance cross the line at *c—c*, coming into a region of low temperature to the left of *c*, cease their violent movements, while those which have started in a different direction keep up their headlong course until they also cross the line *c—c*, which they must inevitably do, purely by the laws of chance, if they continue to be active. Thus in time all the Paramecia which were in the heated region at the right of *c* will have crossed the line *c—c*, leaving the heated space empty. Meanwhile, it is not filled again, because any Paramecia to the left of *c* that may swim toward the heated end, react by reversal of the cilia and turning, when they come to the line *c—c* separating the hot from the cooler region. Thus the Paramecia have entirely vacated the region to the right of *c*, simply by keeping up their undirected movements, until by chance a practicable one carries them across the line. At a certain time after the beginning of the reaction a stream of Paramecia will be seen crossing the line *c—c* from right to left, since any other movement in this region causes a reversal of the cilia and turning.

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[1] MENDELSSOHN, M.: Archiv f. d. ges. Physiol., 1895, lx, p. 1.

At the other end, in the region resting on the ice, the cold does not act like the heat in greatly heightening the activities of the animals; it does, however, stop the thigmotactic reaction, so that all the Paramecia are set in motion, though perhaps very slowly. (If the cold is allowed to act suddenly and strongly, the Paramecia are simply benumbed, and remain where they are.) Some of the Paramecia thus swim by chance across the line *d—d;* the reaction is not, however, so quick and sharp as in the case of too high a temperature, and a number of stragglers will remain benumbed in the cold region. But those which are to the right of *d*, whether originally so, or after having crossed the line *d—d* from the cold region, do not cross this line again, since when they come to the line the cold causes a reversal of the cilia and a turning away. The cold region, therefore, gradually becomes emptied of Paramecia, like the hot space at the opposite end of the slide. The Paramecia in the middle region of the slide may now swim undisturbed anywhere between *c* and *d*, but as soon as they attempt to swim across the line *c—c* or *d—d*, the reaction sets in, and they are turned back. After a time, therefore, practically all the Paramecia are gathered in the optimum region between *d* and *c*, while the ends of the enclosure are empty. The result is not due to direct swimming toward the optimum temperature, nor a direct swimming away from the hot or cold region, but by a sort of " automatic selection " of movements. The Paramecia in the two ends keep swimming hither and thither, until a chance movement in the right direction carries them into the optimum zone. Motion takes place in any direction till one is automatically selected by the fact that it brings the Paramecium into new conditions. The analogy of this to the natural selection of structures is obvious.

Mendelssohn, who described the phenomena of thermotaxis in Paramecium without a knowledge of the finer mechanism of the reaction, assumed that the Paramecia turned and swam *directly* toward the optimum region from either end. In a Paramecium approaching a source of heat, the anterior end would thus be slightly warmer than the posterior end. Mendelssohn assumed that the directive control exercised by temperature must be due to this slight difference in temperature between the anterior and posterior ends of the same animal. He found that in a trough 10 cm. long, a difference of three degrees Celsius between the two ends of the trough was sufficient to cause the Paramecia to collect in the warmer end. The average length of a Paramecium being about 0.2 to 0.25 mm., it was easy to calculate that there was a difference in temperature of about 0.01° C. between the two ends of a Paramecium swimming lengthwise in the trough. It was, therefore, concluded that the Paramecia were sensitive to a difference in temperature of 0.01° C. But, as shown above, the method of reaction is much coarser than that assumed by Mendelssohn; the animal swims from one side of the optimum region to the other, not reacting at all until it comes to a region decidedly hotter or colder. The sensitiveness to temperature differences may, therefore, be much less than that given by Mendelssohn. In a trough with the optimum temperature at one end and changing gradually to the other, the only difference that one can be certain of their appreciating is that between the positive and negative limits of their excursions. If a single Paramecium swims back and forth over a stretch of 5 mm. lying between the cooler and warmer regions of the trough, then it must be concluded that it is sensitive to the difference in temperature between points 5 mm. apart; sensitiveness to any less difference is not proved. Similar considerations apply to other sources of stimuli, since the mechanism of reaction is the same for all classes of stimuli. It is evident that the sensitiveness of Paramecium to different agents may have been greatly overestimated.

Moreover, similar deductions as to the sensitiveness of other organisms must be considered insecure until the exact mechanism of their reactions has been determined.

While the general reaction I have described is evidently from certain standpoints an extremely simple one, yet it bears strongly marked the distinctive features of reactions to stimuli as we know them in the higher organisms, — namely, the lack of any direct and apparent relation between the effect and the cause. In studying the activities of inorganic matter, it is possible to establish relationships of cause and effect — often through a somewhat extended chain from the first cause to the final effect — that may be clearly perceived as causal relations, — so that the effect could be predicted from a knowledge of the first cause and the attendant conditions. On the other hand, in dealing with the activities of living bodies, we are confronted with phenomena in which the steps connecting cause and effect cannot be traced; such phenomena are called reactions to stimuli. Evidently, one of the chief objects in the investigation of the activities of living bodies must be the attempt to resolve reactions to stimuli into the chain of causes of which we must believe them to be composed, so that a series of steps can be traced, each satisfactory from a causal standpoint, from the original cause to the final effect. Can we hope by a study of the simplest unicellular organisms to approach such a resolution of life activities into connected chains of cause and effect? As a hypothetical example of such a resolution may be cited Verworn's theory of the chemotactic movements of Amœba toward oxygen; if this theory is true, the relations of cause and effect are clearly traced from the original cause to the final effect. In the case of the comparatively complicated organism Paramecium, it was perhaps scarcely to be expected that any clear evidence of the actual causal relation between stimulus and reaction should be made apparent, and as a matter of fact, we find, from a causal standpoint, an absolute gulf between them. It is not even possible to trace such general relations between the contractility of the cilia and the nature and position of the sources of stimuli as have been assumed by various authors; Jensen,[1] for example, assumes that a stimulus acting upon one side of the Paramecium causes the cilia of that side to beat more strongly, thus turning the animal away from the source of stimulus; some such relation might perhaps have been reasonably anticipated. But we find that the most varied and even opposite stimuli produce the same reaction, and that stimuli at one end, at the side, or acting over the entire surface of the animal at once, produce the same result. The reaction appears to be purely arbitrary, without any relation to the nature of the cause. It evidently depends upon the internal mechanism of the body of Paramecium, and this mechanism is so complicated that we are quite unable to trace the steps which lead from cause to effect.

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[1] Jensen, P.: Archiv f. d. ges. Physiol., 1893, liii, p. 471.

For all practical purposes, therefore, the reactions of Paramecia present phenomena not a whit simpler for a causal understanding than the activities of a highly developed Metazoan. The mechanism of the unicellular organism, lacking as it does a complex nervous system, may, as a matter of fact, be simpler than that of a Metazoan, but in both cases the mechanism is of sufficient complication to interpose for our understanding an absolute break between stimulus and reaction; hence the study of the Protozoan gives no deeper insight into the essential nature of such reactions than does the study of a Metazoan. Whether the reactions of such a comparatively undifferentiated lump of protoplasm as Amœba will really prove of any more value for such an insight remains to be seen; certainly Amœba is deserving of a thorough experimental study from this standpoint.

Thus the principal intrinsic interest of this investigation lies not so much in the fact that the organism studied is a mass of protoplasm constituting but a single cell, as in the fact that it represents a very low place in the psychological scale. From the psychological standpoint, the reactions of Paramecium are extraordinarily simple. We have in this animal perhaps as near an approach to the theoretical reaction postulated by Spencer and Bain for a primitive organism, — namely, random movements in response to any stimulus, — as is likely to be found in any living organism. The motions are strictly random, so far as the position of the source of stimulus is concerned; the animal always swimming, after stimulation, in the direction of one of its own ends (the posterior), and turning toward one of its own sides (the aboral), without regard to the relation of these directions to the source of stimulus. And by the repetition of the reaction the direction of movement is frequently changed, — always without relation to the localization of the stimulus. It appears not to have been foreseen, theoretically, that such random movements would of themselves if continued carry the animal out of the sphere of influence of the agent causing them and keep it from re-entering. To accomplish this result, it is only necessary that the direction of motion should be changed at the moment when the stimulus begins to act, and at intervals so long as its action continues.

It is evident from this method of reaction that Paramecia are neither directly attracted nor directly repelled by any agencies. It is often assumed that it is a universal property of living organisms to react in two opposite ways toward stimulations, — to be attracted by some and repelled by others. In view of the reactions of Paramecium this assumption must fall to the ground, as certainly not universal, and possibly not nearly so general as has been supposed. The mechanism of the reactions of most simple organisms remains yet to be ascertained.

In regard to the position in the psychological scale to be assigned to Paramecium, the following may be said. The organism responds to any stimulus by a definite, well-characterized reaction. The same may be said of the isolated muscle of a frog. The intensity of the reaction varies with the nature and intensity of the stimulus; this also is true for the muscle. Under certain influences the Paramecium remains quiet; likewise the muscle. The *directive* relations of the motions performed are determined both in the Paramecium and in the muscle by the structure of the organism, not by the localization of the stimulus. There seems, then, no necessity for assuming anything more in order to explain the reactions of Paramecium than to explain the reactions of the muscle. We require, therefore, little or nothing more than irritability, or the power of responding to a stimulus by a definite movement, to account for the activities of Paramecium. Since the direction of motion of the Paramecium has no relation to the position of the source of stimulus, there is no need to suppose that the animal has anything related to a *knowledge* of this position. Moreover, it exercises no *choice*, — the direction of motion being always the same with reference to the parts of the animal. I do not see that we are compelled to assume consciousness or intelligence in any form to explain the movements of this creature.

SUMMARY.

1. Paramecium has a single motor reaction, by which it responds to all classes of stimuli. This reaction consists of the following activities. (1) The direction of the stroke of the cilia is reversed, over the entire surface of the animal, so that they strike forward, driving the animal backward. (2) The reversal ceases; the cilia in the oral

groove strike backward, as at first, while the aboral cilia of the anterior half of the body strike *transversely toward the oral side. This results in turning the animal toward the aboral side.* The anterior end may, if the stimulus is strong, be curved toward the aboral side, thus aiding in turning toward that side. (3) The cilia all strike backward as at first, driving the animal forward again. At the end of the reaction the Paramecium is swimming forward on a path which lies at an angle to the path on which it was moving at first.

2. The intensity and duration of the different parts of this reaction vary with the nature and intensity of the stimulus.

3. Paramecia do not turn directly toward a beneficial source of stimulus, nor directly away from an injurious source of stimulus, but the reaction is always as stated under 1. That is, localized stimuli, acting on one side or one end of the animal, have the same effect as stimuli acting upon the entire surface of the body at once. The direction in which the Paramecium turns after a stimulus is determined by internal factors, and is expressible in terms of the animal's structure; it has no relation to the position of the source of stimulus. The *absolute* direction in which the animal turns is a matter of chance; if it does not carry the animal away from the obstruction the first time, the entire reaction is repeated till it does.

4. *Positive* and *negative taxis*, as applied to Paramecia, are merely convenient terms for expressing the fact that the animals form collections in the regions of certain influences and do not form such collections in others; the terms do not express motor reactions. Paramecia are not directly attracted or repelled by any agencies or conditions. The collections formed in the regions of certain agencies are due to the fact that these agencies cause *no motor reaction.*

5. Different classes of chemical or physical stimuli do not have qualitatively different methods of affecting the stroke of the cilia.

6. In view of the mechanism of the reactions, the sensibility of Paramecium to different agents has probably been much overestimated, since it is not the differential effect on the anterior and posterior ends of the animal that induces the reaction.

7. In the reactions of Paramecium the steps between cause and effect cannot be traced, opposite causes producing the same effect, and the same cause if continued producing opposite effects. The nature of the reaction is conditioned by the internal mechanism of the animal's body.

8. The reactions of Paramecium indicate that the organism stands at the very bottom of the psychological scale. Simple irritability, or the property of responding to a stimulus by a fixed set of movements, seems sufficient to account for its activities.

The results presented in the foregoing paper were obtained in connection with an extended study of the chemotaxis of Paramecium, carried out in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes during the summer of 1898, and continued at Dartmouth College during the following autumn. It is a pleasure to acknowledge here my indebtedness to Prof. J. E. Reighard, the Director of the Survey, and to the officials of the United States Fish Commission for assistance and courtesy in every way in carrying on this investigation, as well as for permission to publish the present paper.

DARTMOUTH COLLEGE,
HANOVER, N. H., Jan. 17, 1899.

ON ABSORPTION FROM THE PERITONEAL CAVITY.

By LAFAYETTE B. MENDEL.

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THERE is abundant experimental evidence to indicate that when an intravascular fluid is separated from an extravascular one of different composition, an interchange of substances will take place between them. Given a separating membrane, *e. g.* a capillary wall, sufficiently permeable to the substances in solution, the two fluids will manifest a tendency to become equalized in composition. Thus substances originally dissolved in the extravascular fluid only may pass into the intravascular solution according to the established principles of osmosis.[1] That substances foreign to the blood can in this way be absorbed into the circulation directly through the blood vessels has long been known.[2] In investigating the absorption of dissolved substances with reference to the channels by which they enter the circulation from the connective tissue spaces, it has been a customary procedure to introduce readily detectable compounds into these spaces, and to watch for their appearance in the blood or lymph stream under various conditions. The outcome of these researches has been to attribute to the blood vessels an important rôle in absorption from connective tissue spaces, quite in accord with the requirements of physical diffusion; although it has by no means been denied that the lymphatics also may be engaged in the transfer from the tissue cavities to the blood.

The problem of absorption from the pleural and peritoneal cavities has been studied by Starling and Tubby[3] among others. They introduced a colored substance — indigo-carmine or methylene blue — in solution into the cavities, and observed the reappearance of the pigment in the urine after ligation of the thoracic ducts, or after introducing cannulas and collecting the lymph before its entrance into the blood stream. In their experiments the urine was collected through a cannula tied into the ureter, "or in most cases into the bladder, either through an opening into the abdominal wall, or through an opening in the urethra, which was exposed by splitting the symphysis pubis." They observed in this way that "5–20 minutes after the injection of the coloured fluid into the serous cavities the urine became tinged with blue, when indigo-carmine was used, or green, when methylene blue was the substance employed. The urine, on shaking with air, rapidly deepened in tint, until the colour was as intense as that of the fluid injected. After a further lapse of time, varying from ten minutes to four hours, the lymph flowing from the thoracic duct also became slightly tinged, but the colour never deepened, on shaking with air, beyond a very light blue or green. The flow of lymph was not increased" (p. 143). The investigators add: "It might be argued from these experiments that the absorption of the colouring matter from the pleural or peritoneal cavity took place by means of the blood vessels and by means of the lymphatics, the former process however being the quicker of the two. It is very doubtful however whether the slight colouration of the lymph which was observed in these experiments is occasioned at all by lymphatic absorption. If methylene blue or indigo-carmine be injected into the blood stream, the lymph flowing from the thoracic duct within half a minute becomes coloured. Now in these cases the colouring matter must have been present in the blood in order to have been excreted by the kidneys, and the colour of the lymph may be caused by a passage of coloured lymph from the blood vessels, not by any direct absorption of the blue from the serous cavities" (p. 144). The data presented thus indicate a direct interchange between the fluid in the cavities and the blood in the vessels. Cohnstein,[1] likewise, has found these phenomena in direct accord with the principles of osmosis.

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[1] Cf. Róth: Archiv für Physiologie, 1898, p. 542.

[2] Cf. Schaefer's Text-book of Physiology, 1898, i, p. 303; Munk, I.: Archiv für Physiologie, 1895, p. 387.

[3] Starling and Tubby: Journal of physiology, 1894, xvi, p. 140.

Comparable with the preceding observations, and indicating the immediate importance of the blood vessels in absorption within the tissues, are experiments by I. Munk.[2] He severed the truncus lymphaticus colli, which conducts away the total lymph from the head in the rabbit, and removed the lymph coming from the cephalic end of the duct. When strychnine was thereupon injected under the skin of the head, no delay in the oncoming or intensity of tetanic symptoms was observed, although the blood vessels formed the only channel of communication with the general circulation. Furthermore, no strychnine could be detected in the lymph collected from the head. Again, Wertheimer and Lepage[1] have demonstrated the active participation of the blood vessels of the liver in the absorption of substances from the bile. When a solution of indigo-carmine was allowed to flow into the ductus choledochus of a dog at a pressure of 30 cm., the urine collected from the ureter was found to be blue some minutes before the lymph flowing from the thoracic duct showed a trace of the pigment. The authors conclude that the lymphatics play a subordinate part, at most, in this process of absorption.[2]

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[1] COHNSTEIN: Centralblatt für Physiologie, 1895, ix, p. 403.

[2] MUNK, I.: Archiv für Physiologie, 1895, p. 387.

In a recent paper Meltzer[3] has reviewed the investigations of Starling and Tubby. Admitting that their experiments "if confirmed, would indeed prove the correctness of the blood vessel theory of absorption," Meltzer has repeated them and has presented the protocols of a series of further experiments which lead him to quite different conclusions. The method of experimentation was essentially that employed by Starling and Tubby. Colored substances, or potassium ferrocyanide, were introduced into the peritoneal cavity; the lymph was collected from the thoracic duct and the urine by means of a catheter introduced into the bladder through an external opening in the urethra. An extensive opening of the abdominal cavity was thus avoided. "In all of the experiments, the coloured fluid or characteristic Prussian blue appeared invariably in the lymph distinctly earlier than in the urine. . . . The lapse of time between the injection into the abdomen and the appearance in the lymph was in all the experiments approximately the same — an average of fifteen minutes. . . . On the other hand the time for the first appearance of the colour, etc., in the urine varied considerably in the different experiments." Meltzer adds: "In the few experiments I made by injecting coloured fluid directly into the circulation, the colour appeared in the lymph before it appeared in the urine; the interval for the lymph was approximately constant, while the interval for the urine was quite variable — between seven and thirty-four minutes."[4] In view of this reverse of the result obtained by Starling and Tubby, Meltzer has rejected the theory that the peritoneal absorption takes place through the walls of the blood vessels and not by way of the lymphatics.

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[1] WERTHEIMER and LEPAGE: Archives de physiologie, 1897, p. 373.

[2] Page 374: "Ces quelques exemples suffisent pour prouver non seulement que les vaisseaux sanguins prennent une part active à la résorption du pigment bleu. mais encore que les lymphatiques n'y ont qu'une part très restreinte."

[3] MELTZER: Journal of physiology, 1897, xxii, p. 198.

[4] MELTZER: *Ibid.* p. 203.

The preceding discussion has led Starling[1] to repeat his earlier experiments with due regard to the precautions emphasized by Meltzer, and he has failed to discover any fallacy in them. He calls attention, however, to the necessity of emptying the bladder at regular intervals of two or three minutes by pressure on the abdominal wall, when the urine is collected through a cannula inserted directly into the bladder. Otherwise the urine is apt to accumulate in the latter and only the overflow is collected; a delay is thus occasioned between the secretion of a portion of urine and its appearance in the cannula. To this point I shall refer again somewhat later.

In the course of some experiments on the action of certain lymphagogues[2] it occurred to me that if colored substances introduced into the abdominal cavity are carried into the circulation mainly through the lymphatics, an acceleration of the lymph flow might be accompanied by a more rapid or extensive transference of the pigment along this channel.[3] The first experiment was on a dog in which an intravenous injection of heteroalbumose had produced pronounced lymphagogic effects together with complete stoppage of urinary flow. Ten c.c. of strong indigo-carmine solution were introduced into the peritoneal cavity. No blue could be detected in the lymph collected within an hour; the bluish tint of a sample of blood serum, however, gave evidence of an active absorption. In another animal previously receiving an intravenous injection of hemipeptone, the influence of which had already disappeared, fifteen c.c. of the blue solution were introduced into the peritoneal cavity. The blue color appeared in the urine in *fourteen* minutes, in the lymph three minutes later. Observations of this kind led to a series of experiments on normal dogs under a variety of conditions. The technique of the experiments was essentially like that adopted by Starling and by Meltzer. The dogs were anæsthetized (after 36 hours' fasting) by a subcutaneous injection of morphine sulphate followed by chloroform-ether administration. Cannulas were tied into the thoracic duct and both ureters. For collecting the urine the ureter method seems to me to be the preferable one, since it prevents any stagnation of fluid in the bladder. But inasmuch as the method has been criticised because it occasions the opening of the abdominal cavity the urine was collected in some cases directly from the bladder, in bitches, by introducing a catheter exteriorly through the urethra. The results obtained were not modified by this procedure, as the protocols will show. The colored fluid (indigo-carmine solution) was always introduced into the peritoneal cavity with a blunt pipette[1] through a small opening in the linea alba near the sternum. The abdominal wounds were all carefully stitched afterwards. The details of some experiments follow.

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[1] STARLING: Journal of physiology, 1898, xxii, p. xxii.

[2] CHITTENDEN, MENDEL, and HENDERSON: This journal, 1899, ii, p. 162.

[3] In this connection it was, of course, remembered that the lymph formed through the influence of these "lymphagogues" has its *origin* primarily in the liver.

I. Dog, 16 kilos. Cannula in thoracic duct and in each ureter. The lymph flow in ten minutes was 2.3 c.c.; urinary flow was good. At 12.30, 20 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. At 12.46 the urine was tinged blue; the lymph continued normal in color until 12.58-12.59, when a faint blue tinge was evident. The urine, at this time, was perfectly dark with blue. At 1.05 the lymph showed a deeper blue, but the color was not intense; at 1.20 the color began to deepen, and still persisted when the experiment was concluded at 2.30. The urine continued to show a very dark blue color.

Thus the color appeared in the urine in *sixteen* minutes; in the lymph in *twenty-eight* minutes.

II. Dog, 20 kilos. Cannula in thoracic duct and in each ureter; cannula for bleeding in femoral artery. The urine and lymph flowed regularly, the latter at the rate of 1.9 c.c. in ten minutes. At 12.22 20 c.c. saturated indigo-carmine solution were introduced into the peritoneal cavity. A bluish tint appeared in the urine at 12.31, becoming very deep at 12.34. The color began to show in the lymph at 12.45 and gradually increased in intensity. At 12.47 a blood sample was drawn; the serum was obtained by centrifugalization and was precipitated with alcohol. The alcoholic filtrate was decidedly blue at a time when the lymph was only beginning to show a trace of color. At 2.30, when the experiment was concluded, the urine was still intensely blue with pigment, while the lymph was light blue.

Thus the color appeared in the urine in *nine* minutes; in the lymph in *twenty-three* minutes.

III. Bitch, 9 kilos. Cannula in thoracic duct. Catheter introduced exteriorly through urethra into the bladder. Lymph flow in ten minutes was

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[1] ADLER and MELTZER caution against the use of a sharp cannula for this purpose, since it may enter the intestinal lumen occasionally: Journal of experimental medicine, 1896, i, p. 493.

1.9 c.c. The urine flowed readily whenever pressure was applied over the bladder; otherwise the flow was not regular, a few drops being expelled occasionally with respiratory movements. During the experiment the urine was expressed at intervals of about three minutes. At 4.01 15 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. At 4.10 the urine was distinctly blue, and the color had become intense at 4.20, when the first bluish tint appeared in the lymph. The color deepened somewhat, but had decreased, if anything, at the conclusion of the experiment at 5.05. A post-mortem examination showed the blue color well distributed throughout the peritoneal cavity.

In this experiment the possibility of escape of color along wounds in the abdomen was excluded. Nevertheless, the color appeared in the urine in *nine* minutes; in the lymph in *nineteen* minutes.

IV. Bitch, 10 kilos. Cannula in thoracic duct; catheter in bladder as in Exp. III. Urine was readily obtained by gentle pressure. The lymph flow in ten minutes was 2 to 3 c.c. At 11.35 2 c.c. indigo-carmine solution were introduced into the peritoneal cavity. At 11.53 the urine showed the blue color, which soon deepened. No trace of blue could be detected in the lymph within an hour. Accordingly at 12.43, 10 c.c. aqueous indigo-carmine solution were introduced in the peritoneal cavity. At 1.0 the urine was decidedly more blue than before; the lymph assumed a bluish tint (?) which became distinct at 1.05, growing deeper and remaining blue when the animal was bled to death at 2.15. Practically all the colored fluid had disappeared from the peritoneal cavity. The diaphragm alone was stained deep blue.

Thus the color appeared in the urine in *eighteen* minutes, while no blue could be detected in the lymph within an hour; and again in the urine *within seventeen* minutes, and in the lymph somewhat later. The experiment is interesting because of the method employed to collect the urine and of the *small amount* of indigo-carmine at first introduced.

The following details are included from additional experiments in which the lymph flow was accelerated by intravenous injection of a lymphagogue previously to the introduction of the colored solution into the peritoneal cavity.

V. Dog, 20 kilos. Cannula in thoracic duct and in each ureter. Cannula for intravenous injection in facial vein. The flow of urine and lymph being found satisfactory, at 11.33–11.34 50 c.c. of 20 per cent sodium chloride solution were infused intravenously. In the succeeding twenty-five minutes 46 c.c. of lymph and 112 c.c. of urine were collected. At 11.44 12 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. Blue color appeared in the urine at 11.48, and rapidly increased in intensity. At 11.53 the lymph assumed a faint bluish tint, which grew more pronounced, but was never very deep. When the lymph flow diminished again, a solution of five grams of Grübler's "pepton" was infused intravenously at 12.15. The urinary flow ceased at once. The lymph continued blue in color, 26 c.c. being collected in the succeeding fifteen minutes.

Thus the color first appeared in the urine in *four* minutes; in the lymph in *nine* minutes.

VI. This experiment has already been referred to briefly. Dog, 15 kilos. Cannula in thoracic duct and in each ureter. A previous injection of hetero-albumose had stopped the urinary flow. The lymph flow in ten minutes was 4 c.c. At 5.0 10 c.c. indigo-carmine solution (in 0.7 per cent sodium chloride solution) were introduced into the peritoneal cavity. At 6.0 the serum obtained from a sample of blood showed a faint bluish tinge. No pigment could be detected in the lymph, which was still flowing steadily.

VII. Dog, 15.5 kilos. Cannula in thoracic duct and in each ureter. Cannula for intravenous injection in facial vein. After a solution of eight grams of Grübler's "pepton" had been introduced into the latter, the urinary flow practically ceased. The lymph flow in ten minutes was 10.6 c.c. Immediately after the infusion of the albumose-peptone 10 c.c. saturated aqueous indigo-carmine solution were introduced into the peritoneal cavity. The lymph continued to flow abundantly; no blue could be detected in it within three hours. A post-mortem examination showed the pigment distributed throughout the abdomen; the thoracic cavity was entirely free from it.

A similar experiment was carried out with analogous results on a dog of 20 kilos. Nearly 50 c.c. of lymph were collected without the appearance of any pigment in it being noted.

The data presented agree closely with those already recorded by Starling. In every case the characteristic color appeared in the urine before it could be detected in the lymph flowing from the thoracic duct, the interval observed ordinarily being about ten or twelve minutes. The reverse results obtained by Meltzer with indigo-carmine solutions are briefly tabulated below for comparison.[1]

Indigo-carmine Experiments of Meltzer.

Weight of Dog.	Amount of fluid introduced.	Time of first appearance of pigment in Lymph.	Urine.
kilos.	c.c.	min.	min.
28	50	8	26
..	500	11	23
18	50	12	40
10	50	13	23
30	50	13	44
22	76	14	80

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[1] MELTZER: Journal of physiology, 1897, xxii, p. 198.

In view of the criticisms of previous investigators, it may be well to point out that the tardy appearance of the pigment in the lymph has not been due to any retarded lymph flow in my experiments; the rate of flow varied ordinarily between 1.9 c.c. to 4.4 c.c. in ten minutes. The results are apparently independent of the size of the animal, and the data obtained by collecting the urine from the bladder agree in every respect with those furnished by the ureter method. The amount of colored solution introduced into the peritoneal cavity in my experiments has been relatively small compared with that used by Meltzer; this precaution has seemed desirable in view of the statement that "the absorption of such small amounts certainly afforded a greater similarity with that of the normal lymph than when the large quantities of fluids used by these other writers were employed."[1] Thus, in their experiments on rabbits, Adler and Meltzer used 1 to 2 c.c. of fluid.

It is not difficult to understand the prolonged absence of pigment from the lymph in those cases where only small quantities are introduced into the peritoneal cavity. The foreign substances, being quickly transferred directly into the blood stream, are at once taken up and eliminated by the kidneys, — as the intravascular injection of pigment also indicates. In this way little material is left to pass into the lymphatic system from the lymph spaces by this relatively slower process, or by subsequent transference from the blood vessels.

It has already been mentioned that Starling and Tubby express doubt regarding the direct absorption of coloring matter into the lymphatics in their experiments, and emphasize the possibility that the coloration of the lymph may be due to a "passage of coloured lymph from the blood vessels." Thus they observed that a colored substance introduced intravenously may appear in the lymph flowing from the thoracic duct within half a minute. So far as I am aware, no other comparable observations are recorded showing so brief an interval. For sodium salicylate, which does not influence the lymph flow, Tschirwinsky[2] has observed from four to seven minutes; Cohnstein[3] has found four or five minutes to intervene after potassium or sodium ferrocyanide solutions are injected, and for sodium iodide the writer[4] has repeatedly observed similar intervals. A few additional data obtained with indigo-carmine are given here: —

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[1] Adler and Meltzer: Journal of experimental medicine, 1896, i, p. 484.

[2] Tschirwinsky: Centralblatt für Physiologie, 1895, ix, p. 49.

[3] Cohnstein: Archiv f. d. ges. Physiol., 1895, lix, p. 509.

[4] Mendel: Journal of physiology, 1896, xix, p. 227.

VIII. Dog, 20 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous injection in right facial vein. Rate of flow of lymph in ten minutes was 2.3 c.c.; of urine was 1.1 c.c. At 11.59 to 12.0 10 c.c. of strong indigo-carmine solution (in 0.7 per cent sodium chloride solution) were infused into the vein. At 12.9 the blue color was distinct in the urine. At 2.45 there was still a good flow of urine and lymph; the latter had not yet shown a trace of blue, while the color of the urine had gradually grown less deep, now showing a light green tint. Therefore at 2.45 a further 20 c.c. of the same indigo-carmine solution were infused into the vein. At 2.55 the urine grew deep blue a second time, and at 3.08 the lymph began to show a faint blue tinge which scarcely increased in color. Lymph flow in succeeding forty minutes was 10 c.c. The dog was killed by bleeding and the thoracic duct showed no blue color in any part of its course.

Thus the color appeared in the urine in *ten* minutes; in the lymph in *twenty-three* minutes.

IX. Dog, 11 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous injection in right facial vein. The dog had been without food or water for two days; 50 c.c. 0.7 per cent sodium chloride solution had been infused slowly into the facial vein. Lymph flow in ten minutes was 3.1 c.c.; urinary flow was 0.9 c.c. At 11.42–11.44 15 c.c. strong indigo-carmine solution were slowly introduced intravenously. At 11.59 the urine was colored blue and quickly grew intense in tint. At 12.05 the lymph showed a faint bluish tint, which grew slightly deeper; but at 12.25 the color was doubtful. Therefore 25 c.c. indigo-carmine solution were again injected, whereupon at 12.33 the lymph again showed the blue tint, which deepened somewhat to light blue.

Thus the color appeared in the urine in *seventeen* minutes; in the lymph in *twenty-three* minutes (or with a larger quantity of colored solution in *eight* minutes.)

X. Dog, 15 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous infusion in left facial vein. Urine and lymph flow well; lymph in ten minutes was 3 c.c. At 12.20–12.21½ 20 c.c. one per cent indigo-carmine solution in one per cent sodium chloride solution were introduced into the vein. At 12.28½ the urine was deep blue; at 12.32 a trace of blue appeared in the lymph and gradually increased somewhat in depth of color. At 1.0 20 c.c. twenty per cent sodium chloride solution tinged with indigo-carmine were infused. The acceleration in the flow of urine and lymph immediately followed. The urine grew less deeply blue; the color was missed entirely in the lymph.

Thus the color appeared in the urine in *eight* minutes; in the lymph in *twelve* minutes.

In similar experiments Meltzer[1] has reported that the color appeared in the lymph before it appeared in the urine. Detailed protocols of the experiments have not been published, and few definite statements are made with regard to the urinary flow in these observations, or in those already tabulated. It is difficult to resist the conclusion that the differences between our results are perhaps attributable to variations in the method of collecting the urine. Thus in Meltzer's experiments on direct intravenous injection of colored fluid, intervals of *seven* to *thirty-four* minutes elapsed before color was detected in the urine; with intraperitoneal injections intervals of *twenty-three* to *eighty* minutes are recorded. The results were apparently independent of the size of the animal used. The longest interval that I have observed was *eighteen* minutes in Exp. IV when only 2 c.c. indigo-carmine solution were introduced. Furthermore the actual amount of pigment appearing in the lymph was in no case equivalent to more than a very small fraction of the pigment introduced into the animal, or found in the urine. A more satisfactory explanation does not suggest itself at present; there are, however, quite different and perhaps more satisfactory ways of arriving at an answer to the main problem concerned.[2]

The experiments recorded in this paper are thus in no way opposed to the "blood vessel theory" of the absorption of foreign substances from the peritoneal cavity. Rather they conform with the results which are demanded by the conditions and principles discussed at the outset.

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[1] Meltzer: Journal of physiology, 1897, xxii, p. 203.

[2] In a recent paper on the absorption of various intraperitoneal fluids when the lymph channels are excluded (in the rabbit), Róth concludes: "Dabei findet eine langsame Resorption der isotonischen Lösung durch die *Blutgefässe* statt." Archiv für Physiologie, 1898, p. 545. The older similar experiments by Hamburger have been criticised by Adler and Meltzer: Journal of experimental medicine, 1896, i, p. 482.

THE ORIGIN OF THE "TRAUBE" WAVES.

By HORATIO C. WOOD, Jr.

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RHYTHMICAL variations of blood pressure not associated with any perceptible alteration in the cardiac or respiratory function have been described by several observers. These waves differ more or less in their form and in the circumstances under which they are found. Traube,[1] who was the first to investigate the subject, mentions two types occurring in dogs in whom the artificial respiration had been suspended; the one is distinguished by a rhythm about that of natural respiration and resembles closely the respiratory variations in blood pressure; the other is much larger and slower. Mayer[2] observed waves of the latter class in rabbits either breathing naturally or supplied with artificial respiration. They appeared singly, at irregular intervals, or in rhythmical groups. Their cause was not apparent.

Since all forms of these waves are altered in their character in the same manner by similar influences the conclusion seems probable that the same cause underlies them all. When we come to look for this cause we find in the first place that the waves are due to changes in the vasomotor centre. The evidence in support of this is so universally acknowledged that I will mention only the experiment of Mayer, in which the temporary loss of function of the medulla produced by shutting off the blood supply causes the waves to disappear. The question as to what impels the vasomotor centre to send out these rhythmical impulses is not so easily answered. Hering[3] suggested that impulses pass over from the respiratory centre (irradiation). He grounds his belief on the correspondence in rate between the Traube waves and the respiration, and on the fact that sometimes in imperfectly curarized animals slight spasmodic movements of the diaphragm indicating activity of the respiratory centre, but not strong enough of themselves to have any effect on blood pressure, are seen together with the Traube-Hering waves. The first of these reasons seems hardly applicable at least to the waves described by Traube as recurring at intervals of thirty seconds (Hering himself says the waves recur at the rate of 5 to 15 per minute). Concerning the second argument, slight spasmodic movements may often be observed in the limbs and trunk muscles of imperfectly curarized animals, and Knoll[1] has seen them recur at regular intervals, but they are far too slight to influence the blood pressure.

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[1] TRAUBE: Centralblatt für die medicinische Wissenschaften, 1865, p. 881.

[2] MAYER: Sitz.-Ber. d. Kaiserl. Akad. d. Wissensch. zu Wien, Math.-nat. Kl., 3, 1876, lxxiv, p. 281.

[3] HERING: *Ibid.*, 1870, lx, p. 829.

Mayer supports Hering's theory of irradiation from the respiratory centre on the grounds that any manipulation, as section of the vagi, which lowers the respiratory rate makes the waves more prominent; that stimulation of the central end of a cut vagus alters their type; and that they do not occur after paralysis of the respiratory centre through shutting off the blood supply to the brain. But these reasons are open to objection. Unless the lowering of the rate of breathing is accompanied by a corresponding increase in the depth of each respiratory movement — and the maintenance of the normal respiratory exchange after section of the vagi is doubtful — the accumulation of carbon dioxide should favor the occurrence of these waves; moreover, the vagi are concerned with the innervation of so many functions that to draw any positive conclusions regarding such an obscure subject from the effects of their section is hardly wise. The support drawn from the discovery that stimulation of the central end of the vagus affects the form of the wave, loses much of its weight in view of the fact that Knoll has since found that stimulation of any sensory nerve has the same effect. As to the third reason for his belief, Mayer seems to have forgotten that in occluding the blood supply of the medulla he paralyzed the vasomotor as well as the respiratory centre.

A better method of determining whether the respiratory centre is to be considered as a casual factor in the production of Traube-Hering waves would seem to be the use of some agent which paralyzes the respiratory and not the vasomotor centre. In some experiments with veratrum viride[2] I find that this substance offers us a means of thus separating the two functions. By proper doses (about 0.04 c.c. of the fluid extract per kilo, for dogs) the respiration can be absolutely paralyzed while the rise of pressure brought about by the asphyxia demonstrates that the vasomotor mechanism is still functional. Under these circumstances I have seen the most marked rhythmical variations in the blood pressure, both when it was high (150 mm. Hg) and when it was low, either with artificial respiration or when the animal was asphyxiated. The ***occurrence of Traube waves when the respiratory centre is paralyzed shows that the impulses which occasion their discharge do not arise in that centre.***

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[1] KNOLL: *Ibid.*, 1886, xcii, p. 447.

[2] Veratrum album has the same effect.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. IV.—LAWS OF CHEMOTAXIS IN PARAMECIUM.[1]

BY H. S. JENNINGS.

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1. INTRODUCTION.

IN the second paper of this series of Studies,[2] I have given an account of the mechanism of the reactions of Paramecium that requires a revision (for this animal) of the usual conceptions conveyed by the term chemotaxis. It was there shown that Paramecium reacts in essentially the same manner to all effective stimuli, and that the direction of motion in the reaction has no relation to the position of the source of stimulus, so that the animals are not directly attracted or repelled. When the animals are stimulated in any way, they swim backward, turn toward the aboral side, then swim forward over the new path so determined, without regard to the position of the stimulus. The terms positive and negative chemotaxis thus lose their content almost entirely, at least in the construction which is often put upon them. Nevertheless it will be convenient to employ these terms to express the fact that the Paramecia do, in the manner detailed in the paper above referred to, form collections in certain regions (positive taxis), and leave other regions empty (negative taxis). These phenomena retain their biological significance in full, whatever the means by which they are brought about. In view of the mechanism of the reactions, the following definitions may be given. Certain substances in solution set up in a Paramecium random movements, the direction of movement being changed at the time the stimulus begins to act and frequently so long as it continues to act. As a result of these undirected motions the Paramecium is in time, by the laws of chance, carried out of the region of the substance causing them, and prevented from re-entering. Hence toward these substances it may be said to be negatively chemotactic, and the substances may be called repellent. Other substances cause no motor reaction whatever, so that the Paramecia tend to come to rest within them, and these substances are further of such a nature that they throw the Paramecia into a peculiar physiological condition, such that coming in contact with a fluid not containing these substances causes the motor reaction above characterized, so that the animals are returned into the region of the substance causing no motor reaction. Hence if this region is small a dense assemblage of Paramecia may be formed within it. Substances of this character may therefore be said to be attractive, and the Paramecia are positively chemotactic toward them. The terms repellent and attractive, negative and positive chemotaxis, as used in this paper, are then to be understood to have significations in accordance with the above definitions.

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[1] Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard (Ann Arbor, Mich.), under the auspices of the U. S. Commission of Fish and Fisheries, No. 2. Published by permission of the Hon. George M. Bowers, Fish Commissioner.

[2] The following papers have appeared in the series of Studies of which this is the fourth: I. *Reactions to Chemical, Osmotic, and Mechanical Stimuli in the Ciliate Infusoria*, Journal of physiology, 1897, xxi, pp. 258–321. II. *The Mechanism of the Motor Reactions of Paramecium*, This journal, May, 1899, ii, pp. 311–341. III. *Reactions to Localized Stimuli in Spirostomum and Stentor*, American naturalist, May, 1899, xxxiii, p. 372. These papers will be referred to in the following as I, II, and III, respectively.

2. RELATION OF CHEMOTAXIS TO BENEFIT OR INJURY.

The present paper deals with the nature of the substances thus causing or failing to cause the motor reactions, and with the laws of their action. The first question which will be taken up is in regard to the relation of chemotaxis to the benefit or injury to the organism, caused by the given substance. As shown in II, the effect of the motor reaction is on the whole to carry the organism out of the sphere of action of the substance causing the reaction and to prevent its returning. Is the production of this reaction due to the fact that the substance causing it is injurious to the Paramecia? This is a question of some interest in view of a common theoretical explanation of the origin of chemotactic movements. It is said that through natural selection such individuals as are repelled by injurious substances would survive and perpetuate their kind, while those not repelled by these substances would be destroyed; the converse being true as regards beneficial substances. We might then expect to find that the individuals which now survive have become accurately adjusted in these matters, being repelled by any substance which injures them. Any injury might be conceived to result in the " organic analogue of pain," causing thus motor reactions which take the animal away from the source of injury.

We know already from the first of these Studies that the substances which cause the motor reaction most markedly, and hence give most clearly the phenomena of negative chemotaxis, are chiefly alkalies, while substances in which the Paramecia collect, giving the motor reaction only when they attempt to pass *out* of them, are substances having a weak acid reaction. Thus the distinction between repellent and attractive substances is, to a certain extent at least, based upon purely chemical differences. But it was shown in the same paper that many neutral salts likewise cause the reaction, so that the acid or alkaline character of the solution is not the only determining factor; further, that when acid solutions reach a certain strength, they too cause the same motor reaction as do other substances; it is only weakly acid solutions that are attractive, while stronger acid solutions are repellent. It is a well-known fact that organisms are often thus negatively chemotactic toward substances to which in a weaker solution they are positively chemotactic.

Now, what is the basis of this change of reaction with a change in the strength of solution? The solution of course becomes more injurious as it becomes stronger, and the answer to this question which lies nearest is that the negative chemotaxis of the organisms is due to the injuriousness of the solution. May we say that as soon as an acid solution becomes equally injurious with the repellent alkaline solution, it too becomes repellent? And can we generalize the answer and say that the animals are throughout negatively chemotactic to certain substances in virtue of the fact that these substances are injurious to them?

If the injurious quality of the substance is the determining factor in causing the reactions which result in negative chemotaxis, then evidently two substances which are equally injurious must have equal powers of repelling Paramecia, and if one substance is injurious in a weaker solution than a second, then also must the first substance be repellent in a weaker solution than the second. In other words, the repelling powers of the two substances must be proportional to their injurious effects.

This gives a method of testing the question. We may determine the weakest solutions of two or more substances that repel the Paramecia, and note the ratio of their strength; then determine the weakest solutions of the same substances that will kill the Paramecia in a given time, noting their ratios as before. If the two ratios are approximately the same — that is, if the repellent powers are proportional to the injurious effects — then the evidence is in favor of the proposition that the production of the motor reaction resulting in repulsion depends upon the injurious effect of the substances; if the ratios are not the same, then the relation between repellent power and injuriousness is not one of direct dependence.

I have compared in this way a considerable number of substances. The repellent powers of different chemicals were tested in the manner described in I and II for studying chemotaxis, — by introducing with a pipette drawn to a capillary point a drop of the substance beneath the cover-glass of a slide of Paramecia. The substances to be tested were dissolved in proper proportions in distilled water. In distilled water alone the Paramecia form collections (as shown in I), so that the repellent powers of the solutions are due wholly to the dissolved substance. For finding approximately the weakest solution of a substance that causes repulsion, it is convenient to proceed as follows: A series of covered watch-glasses are filled with solutions of the given substance, each successive watch-glass with a solution one half or one fourth as strong as that in the preceding one. Thus we shall have perhaps the following solutions: $\frac{1}{5}$, $\frac{1}{20}$, $\frac{1}{80}$, $\frac{1}{320}$, and $\frac{1}{1280}$ per cent, etc. The Paramecia are placed on a slide beneath a long cover-glass supported at the ends by bits of glass tubing. Now with the capillary pipette drops of solutions of different strengths are introduced beneath the four corners of the cover-glass, — under one corner a drop of $\frac{1}{5}$, under the next $\frac{1}{20}$, then $\frac{1}{80}$, and $\frac{1}{320}$ per cent. We shall probably find that some of the drops remain empty, while others are quickly filled with Paramecia. Suppose that the $\frac{1}{5}$, $\frac{1}{20}$, and $\frac{1}{80}$ per cent remain empty, while the $\frac{1}{320}$ per cent is quickly filled. It is thereupon evident that the weakest solution which repels the Paramecia lies somewhere between $\frac{1}{80}$ and $\frac{1}{320}$ per cent. By making proper intermediate grades between these two, approximately the weakest solution that repels the Paramecia can usually be determined very expeditiously. All the precautions insisted upon in I as necessary for carrying on such experiments were of course rigorously observed.

I will present in detail the results in two typical cases, then summarize my results on the sixty-five substances tested, in the form of a table.

For a first case we may take two chemically related substances, toward both of which the Paramecia show positive chemotaxis when the solutions are very weak. Chromic acid and potassium bichromate fulfil these conditions. These substances both have an acid reaction, due to the same component in each case, and the Paramecia gather in weak solutions of both of them. In stronger solutions both cause the motor reaction which results in repulsion. Is this due in each case to the injurious effect of the stronger solutions?

For potassium bichromate the weakest solution that repels the animals is about $\frac{1}{20}$ per cent. For chromic acid the weakest solution which sets the motor reaction in operation is about $\frac{1}{150}$ per cent. The ratio of repellent powers is therefore 7.5 to 1 in favor of the chromic acid.

The injurious effects of the solutions are now tested as follows: Watch-glasses with solutions of different strength are arranged in series, as above described, and into each a drop of water swarming with Paramecia is introduced and quickly mixed. The solutions are made in such a way that after the introduction of the water containing Paramecia they are of the recorded strength. The weakest solution in which all the Paramecia are killed in one minute is thus determined for the two substances.

For the potassium bichromate it is found in this way that the weakest solution which kills the Paramecia in one minute is a one per cent solution, — that is, a solution twenty times as strong as the weakest solution that repels them. In the chromic acid they die somewhat more quickly in a $\frac{1}{150}$ per cent solution, — a solution of the *same* strength as the weakest which causes repulsion. The ratio of the injurious effects of the two substances is therefore as 150 to 1 in favor of the chromic acid.

Comparing the two ratios, we find that they are strikingly unequal. While chromic acid has but 7½ times the repellent power of potassium bichromate, it is 150 times as injurious. Potassium bichromate repels in a strength $\frac{1}{20}$ of that which is severely injurious; the chromic acid does not repel until it has reached a strength which is already destructive. In the latter case the repellent power is evidently due directly to the injurious effects; repellent power and injurious effects appear at the same point as we pass from weaker to stronger solutions. If the conduct of the Paramecia is observed under the microscope as they swim backward after coming in contact with a drop of $\frac{1}{150}$ per cent chromic acid, it is at once evident that they are decidedly injured, and frequently they die after swimming backward a little distance. The motor reaction is thus not set in operation by the chromic acid until the Paramecia are already injured and it is too late to save them. When such a drop is introduced beneath the cover-glass, the Paramecia are therefore killed by scores and the drop is soon surrounded by a ring of the dead animals. If a drop of a solution somewhat weaker than $\frac{1}{160}$ per cent is introduced, the Paramecia enter it directly, forming a dense gathering, and after a time it may be observed that many individuals of the group are dead or severely injured. In the case of the potassium bichromate, on the other hand, the Paramecia are never injured whatever the strength of solution used; they gather in a ring about a drop of strong solution, but do not enter it deeply enough to be injured.

For these two substances then the test results negatively; potassium bichromate has twenty times the repellent power it should have if repellent power depends alone on the injuriousness of the solutions.

We may next test two substances which do not attract the Paramecia in any strength, and which are not injurious in virtue of their chemical properties at all, but only as a result of their osmotic pressure. As such substances we may select sodium chloride and cane sugar. Repeated comparative tests with isotonic solutions of these two substances indicate that neither is immediately injurious to the Paramecia except through osmotic pressure; strong isotonic solutions of the two substances kill the Paramecia in approximately the same period of time. Thus if Paramecia are introduced at the same time into 1 per cent sodium chloride solution and 9.64 per cent cane sugar (which is isotonic with 1 per cent solution of sodium chloride), in both cases nearly all the individuals are found to be dead twenty minutes after they were introduced, and in neither solution does the proportion of dead markedly preponderate. This result for Paramecium differs from that gained by True[1] for Spirogyra; in the case of Spirogyra sodium chloride was found to have a distinct toxic effect in addition to its osmotic properties.

The weakest solution of sodium chloride which repels the Paramecia is from $\frac{1}{10}$ to $\frac{1}{20}$ per cent. The weakest solution of cane sugar which sets the motor reaction clearly in operation is 10 per cent. Taking the repellent solution of sodium chloride as $\frac{1}{10}$ per cent, the repellent power of sodium chloride is to that of cane sugar as 10 to $\frac{1}{10}$, or as 100 to 1. On the other hand, as stated above, 1 per cent sodium chloride has the same injurious effect as 10 per cent cane sugar, so that the injurious effects of sodium chloride are to those of cane sugar as 10 to 1. The repellent power of the cane sugar, like that of the chromic acid, is plainly due to its injurious properties. The motor reaction is not set in operation until plasmolysis has begun, as shown by the evident shrinkage of the bodies of the animals, and it is usually too late at this time to save them from destruction. If a drop of strong sugar solution is introduced into a slide of Paramecia, it soon becomes filled with the dead Protozoa. On the other hand a drop of sodium chloride is not destructive whatever its strength, since the motor reaction is set in operation long before the Paramecia have entered deeply enough to be injured. Sodium chloride has, as shown by the ratios given above, ten times the repellent power that it should have if repellent power depends entirely upon injurious effects.

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[1] TRUE: Botanical gazette, 1898, xxvi, p. 407.

A large number of compounds, chiefly inorganic, were tested as to the relation between repellent power and injurious effects, and it was found that the cases given above are typical for all. We can divide chemical substances as regards this matter into two classes, as shown in the following table. In the first column may be placed together substances in which, as in potassium bichromate and sodium chloride, the repellent power is great in proportion to the injurious effects. These substances all set the motor reaction in operation long before any injury has taken place, so that the Paramecia are not harmed in the least when a drop of one of them is introduced into a slide preparation of the infusoria. In the other column may be placed substances in which, as in chromic acid and cane sugar, the repellent power is slight in relation to the injurious effects ; the motor reaction in all these substances is set in operation only when the Paramecia are already injured and it is too late to save them. A drop of any one of these substances introduced beneath the coverglass of a slide preparation of Paramecia proves exceedingly destructive.

We may for convenience classify the substances in the table also into those which are attractive to the animals in weak solutions (A), and those which are repellent in all effective solutions (B).

TABLE I.

1. Repellent power strong in proportion to injurious effects: chemotaxis protective.	2. Repellent power very weak in proportion to injurious effects: chemotaxis not protective.
(A. *Attractive Substances.*)	
Sodium fluoride, Potassium bichromate, Ammonium bichromate, Potassium ferricyanide. (KBr), (KCl), (RuCl), (CsCl).[1]	HF, HCl, HBr, HI, H_2SO_4, HNO_3, Acetic Acid, Tannic Acid, Picric Acid, Chromic Acid, Potassium fluoride, Potassium permanganate, Ammonia alum, Ammonio-ferric alum, Chrome alum, Potash alum, $CuSO_4$, $CuCl_2$, $Cu(C_2H_3O_2)_2$, $ZnCl_2$, $HgCl_2$, $AlCl_3$.
(B. *Repellent Substances.*)	
LiCl, NaCl, (KCl), (RuCl), (CsCl),[1] LiBr, NaBr, (KBr), RuBr, LiI, NaI, KI, RuI, Li_2CO_3, Na_2CO_3, K_2CO_3, $LiNO_3$, $NaNO_3$, KNO_3, NaOH, KOH; $KBrO_3$, NH_4F, NH_4Cl, NH_4Br, NH_4I, $CaCl_2$, $SrCl_2$, $BaCl_2$, $Ca(NO_3)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$.	Cane sugar, Lactose, Maltose, Dextrose, Mannite, Glycerin, Urea.

From this table and the foregoing discussion it is evident that the question proposed is to be answered in the negative. The injuriousness of a substance is not the determining factor in its repellent power, since the repellent power of different substances is not at all proportional to the injuriousness of their solutions. In the substances of the second column, such power as they have to set in operation the motor reaction of Paramecium is indeed evidently due directly to the injuries caused, though this power is nothing like so precise and strong as in the substances of column 1, not being sufficient even to prevent the Paramecia from entering and being destroyed.

The fact that the injuriousness of a solution is not the chief determining factor in its repellent power seems to indicate that the common theory, according to which the reactions of organisms have been fixed by natural selection through the destruction of the individuals not repelled by injurious substances, does not contain the whole truth.

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[1] In the case of the substances enclosed in parentheses, the reactions of Paramecia from different regions varied, as described in a subsequent part of this paper.

Probably many of the substances experimented with have never been met by the Paramecia in the course of their evolution, and their reaction toward these must be determined by other laws. Possibly their reactions are adjusted by natural selection to some small number of substances, while to other substances they react by analogy as it were, — by the similarity of their effects to those of the substances to which the reaction has been adjusted by natural selection. Thus it may be that repulsion for some alkali plays an important part in the biology of the animals, and that the reaction toward other substances of this sort is due to their similarity to the primary one. We know that to the tendency to collect in weak solutions of carbon dioxide is due the apparently gregarious habit of the Paramecia, and this probably serves a useful purpose somewhere in the life of the animals. It may be that the tendency to gather in other acid solutions, even such destructive ones as sulphuric acid and copper sulphate, is a sort of reflection of this tendency to collect in carbonic acid, and that natural selection has had no opportunity to correct this tendency directed toward injurious substances. That there is much opportunity for natural selection to act is clear when one tests any chemical substance by bringing a drop of it among a large number of Paramecia. There is always a number of individuals who do not give the typical reaction, some being much more and some much less sensitive than the majority.

Another consideration of importance is the following: It may not be at all the *direct* effects of a substance on the Paramecia, through which natural selection has adjusted the reaction toward that substance. Thus it may be that in solutions of a certain nature, harmless in themselves, the Bacteria which form the food of the Paramecia are never found; natural selection would then act by starving such Paramecia as were not repelled by these solutions.

3. RELATION OF CHEMOTAXIS TO CHEMICAL COMPOSITION.

If the injuriousness of substances is not the determining factor in their repellent power, what *is* the determining factor? An examination of the table above given brings out the following facts. All the substances in which the repellent power is relatively great contain a large proportion of some metal of either the alkali group (Li, Na, K, Ru, Cs, or NH_4) or of the earth alkali group (Ca, Sr, Ba). This is true of all, whether having an acid, a neutral, or an alkaline reaction. On the other hand most of the substances in the second column contain none of these elements. It is true that certain of these compounds, as potassium fluoride, potassium permanganate, and the alums do contain members of the alkali group. But in potassium fluoride the qualities of the compound are due almost entirely to the fluorine component, as is shown by the fact that this salt, like hydrofluoric acid itself, attacks glass energetically. In potassium permanganate and the alums the proportion of alkali metal is small, as is shown by their formulas. Thus the formula for potash alum is $KAl(SO_4)_2, + 12\ H_2O$, so that in the molecule composed of forty-eight atoms but one of these is K; the properties of these compounds are very little due to the alkali component. The proportion of alkali metal is very much larger in the substances of the first column. This dependence upon the presence and proportion of an alkali metal is in practice so striking that in working with salts whose action on the Paramecia is not yet known, it is usually possible to predict with certainty from the chemical formula into which column they will, upon experiment, be found to fall.

The results on these sixty-five compounds appear then to justify the following conclusion: Compounds which contain a large proportion of one of the alkali or earth alkali metals set in operation the motor reaction of Paramecium even when exceedingly weak; their repellent power is therefore great in proportion to their injurious effects, and the infusoria are not endangered by the presence on the slide where they are swimming of a drop of a solution of these compounds. Substances which do not contain a large proportion of one of these metals as a rule set in operation the motor reaction of Paramecium only when the animals are already injured; their repellent powers are very weak in proportion to their injurious effects, and a drop of one of them introduced into a preparation of Paramecia is very destructive to the latter.

The fact, however, that the injurious properties of a substance on reaching a certain intensity of action do produce the motor reaction is worthy of special notice; in all the substances in the second column the reaction is manifestly due directly to the injuries inflicted, without regard to the nature of the substance. Paramecium is not sufficiently sensitive to injuries to make the reaction thus produced of any protective value, at least under experimental conditions. But it may easily be conceived that this sensitiveness might be so increased that the reaction would be of the greatest importance for preserving the animals; it is probable that in many organisms this condition is realized. We seem to have in Paramecium the very lowest step in the production of reactions due to the "organic analogue of pain" (to use a phrase employed by J. Mark Baldwin), — a step so low that the power of thus reacting seems to have no value for the life of the organisms.

The chief determining factor in the repellent power of solutions is then, for Paramecium, of a chemical nature, with little relation to the comparative injuriousness of the solutions.

The conclusion above drawn as to the repellent power of the alkali metals is reinforced and extended by certain results gained in a study of the compounds of these metals with the halogens, — compounds of lithium, sodium, potassium, rubidium, and cæsium on the one hand, with fluorine, chlorine, bromine, and iodine on the other. These form a definitely circumscribed group of compounds, the characteristics of which, as is well known, vary in many respects with the molecular weights of the elements composing them. A qualitative and quantitative study of the reactions of the Paramecia toward these compounds was begun at Put-in-Bay Island, Lake Erie, during the summer of 1898, in the hope that such a study would throw light on the essential nature of chemotaxis. The work was not finished at Put-in-Bay, and on taking it up again at Hanover, N. H., it was found that the Paramecia here gave in part different reactions from those studied at Put-in-Bay. The differences are of such a nature as to be not unintelligible, yet it will be necessary to consider separately the results obtained at the two places.

The elements belonging to the group of halogens may be arranged in a series, beginning with that having the least atomic weight and proceeding to that which has the greatest atomic weight, as follows: fluorine (at. wt. 19), chlorine (at. wt. 35), bromine (at. wt. 80) iodine (at. wt. 127). As is well known, this is also the order of the chemical activity of these elements, the lightest being most active. In the same way the alkali metals may be arranged in a series beginning with the lightest, as follows: lithium (at. wt. 7), sodium (at. wt. 23), potassium (at. wt. 39), rubidium (at. wt. 85), and cæsium (at. wt. 133). This series also gives in a general way the order of chemical activity of the elements, the lighter ones being more active.

The compounds of these two sets of elements may be arranged in a table in such a way as to give the halogen series from above downward, the alkali series from left to right. In the ensuing table, arranged in this manner, are placed only the compounds which I was able to procure and use for studying the reactions of the Paramecia at Put-in-Bay. For convenience the hydroxides of the metals and the acids of the halogens are brought into the table, — the former constituting the lower row, the latter the right-hand column.

TABLE II.

	Li(7)	Na(23)	K(39)	Ru(85)	Cs(132)	
F(19)	....	NaF	KF	....	....	HF
Cl(35) . . .	LiCl	NaCl	KCl	RuCl	CsCl	HCl
Br(80) . . .	LiBr	NaBr	KBr	....	....	HBr
I(127) . . .	LiI	LiI	KI	....	....	HI
		NaOH	KOH			

In this table, as will be observed, the compounds of the lighter (and more active) metals are placed to the left; the compounds of the lighter (and more active) halogens in the upper row. In the right-hand upper part of the table are given therefore compounds of light, active halogens with heavy, inactive metals; in these the influence of the halogens might be expected to predominate. In the left-hand lower corner are given compounds of light, active metals with heavy, inactive halogens; in these the metal component might be expected to predominate.

Now some of these substances repel the Paramecia in any effective concentration; that is, they produce the motor reaction which results in carrying the Paramecia outside of their influence; these we may call the repellent substances. Others when weak do not cause the motor reaction when the Paramecia enter them, but when the animals attempt to swim *out* of the drop into the surrounding water the motor reaction occurs, and the Paramecia return into the drop. They thus form collections either within such drops or surrounding them in a close ring, — in the manner described in I, p. 269. These we may call attractive substances.

All the attractive compounds in the above table are underscored; those not underscored are repellent. It will be seen, first, that all the acids or hydrogen compounds of the halogens are attractive, the hydroxides, or hydroxyl compounds of the metals repellent. Further, that all the compounds of the lightest, most active halogen (fluorine) are attractive. Of the next lightest, chlorine, three salts are attractive and two repellent. Of the heaviest, most inactive halogen, iodine, all the salts studied are repellent. Of the lightest, most active metal, lithium, all the salts tested are repellent, and the number of repellent salts decreases as we pass toward the heavier metals. Thus the compounds of light, active metals with heavy, inactive halogens are repellent; those of light, active halogens with heavy, inactive metals are attractive. A diagonal line can therefore be drawn, as shown in the table, from the upper left hand to the lower right hand, separating all the attractive substances on the right from all the purely repellent substances on the left.

It thus appears that the Paramecia react to these compounds with relation to the elements of which they are composed. The metal components tend to repel the Paramecia; the halogen components to attract them. The acids of the halogens, in which the halogen may be presumed to give character to the compound almost alone, do not repel except in such solutions as to be immediately destructive, as previously discussed. The hydroxides, or bases, in which the metals give the character to the compound, are repellent in any solution strong enough to produce any effect whatsoever. Those compounds which are partly metal and partly halogen are attractive or repellent in accordance with whichever one is the predominant element.

The same conclusion is reached by a study of the exact method of behavior of the Paramecia toward the substances above classed as attractive. Toward these compounds the infusoria are as a rule ***both attracted and repelled.*** As just stated the acids are purely attractive, the bases purely repellent. Toward potassium fluoride, in which the halogen component is so active as to attack glass even in this salt, the animals react as toward a pure acid. Toward sodium fluoride, in which the fluorine is so bound by the sodium that the two produce a comparatively inactive salt, the Paramecia are both strongly attracted and strongly repelled. When a drop of one per cent sodium fluoride solution, for example, is introduced into a slide of Paramecia, it will soon be found that the animals have gathered in a dense ring about the drop, leaving its centre entirely free. In this ring they swim back and forth, giving the motor reaction whenever they come to the inner side of the ring, where the stronger sodium fluoride is, or when they come to the outer side of the ring, where pure water is. Sodium fluoride thus gives a striking example of apparently positive and negative chemotaxis to the same substance. Of compounds outside the group at present under consideration, potassium and ammonium bichromate and potassium ferricyanide show precisely the same phenomena.

The other salts classified in the table as attractive show the same phenomena as sodium fluoride, except that the attraction is less strong and the repulsion stronger. The Paramecia gather in a narrow ring about them, and this ring contains at a given time comparatively few individuals, though enough to make the ring evident as a rather sharply defined white line around the drop. If the individuals constituting this ring are closely observed, it becomes evident that the motor reaction is set strongly in operation when they come to the inner boundary of the ring, where the stronger solution of the salt is found, and much less strongly when they come to the outer boundary, against the ordinary water. Thus many individuals remain in the ring but a short time, finally crossing the outer boundary and escaping, so that the ring does not increase continually in numbers and size, as it does in the case of sodium fluoride; it consists of changing individuals, each one being detained usually but a short time. In some of the salts the ring thus formed is almost evanescent: for a short time it will be distinct, then almost disappear, then reappear again. Finally, about the salts which are not underscored in the table no ring of Paramecia is ever formed.

This formation of a ring of greater or less extent about solutions of various salts is a very curious phenomenon. The Paramecia are evidently thrown into such a condition by a very weak solution of the substance that the contact with the surrounding water now acts as a shock or stimulus to set the motor reaction in operation, — while this motor reaction is also induced by a stronger solution of the same salt, such as is found nearer the centre of the drop. In view of the facts adduced, it seems apparent that the shock on reaching again the outer water is due to the physiological effect of the halogen component, as this shock alone is produced in compounds in which, as in acids and potassium fluoride, the halogen is the element that chiefly gives character to the solution. On the other hand, the stimulus due to entering the salt itself is a result of the metal component, since this stimulus alone is produced in those compounds in which, as in hydroxides (and carbonates) the characteristics of the compound are due chiefly to the metal.

The above results were obtained at Put-in-Bay in a series of about 1000 tests of the substances in the table. The compounds used were tested many times, dissolved both in distilled water and in the same water in which the Paramecia were found, with every possible precaution to secure accuracy, and with uniform results as given above. But on continuing the series of experiments at Hanover, N. H., with the intention of filling the gaps in the table as above given, different results were obtained with the Paramecia of this region for certain of the salts. Toward KCl, KBr, RuCl, and CsCl the Paramecia of Hanover show only repulsion, without any sign of the formation of a ring about them, which was so evident at Put-in-Bay. Toward the NaF and KF on the other hand they react exactly as did those at Put-in-Bay, forming a ring about the NaF and reacting toward the KF as toward an acid. Toward all other members of the above table the Hanover Paramecia likewise react exactly as did those at Put-in-Bay. This variation is evidently not of a character to modify in any way the conclusions to be drawn. The Paramecia at Hanover simply fail to show a very faint reaction given by those from Lake Erie in the presence of certain substances; they seem therefore merely slightly less sensitive; in other respects the reactions are similar in all the Paramecia studied. The Paramecia at Put-in-Bay were cultivated in lake water containing decaying water plants, chiefly Nuphar, Naias, and Ceratophyllum. At Hanover the Paramecia came from cultures of hay, in water from small ponds of the neighborhood. Possibly the difference in the culture medium has something to do with the difference of reaction in the Paramecia of the two regions.

The reactions toward certain other substances may be stated briefly. Toward the carbonates of lithium, sodium, and potassium the reaction is the same as toward the hydroxides of these metals. Also toward the nitrates of these metals and toward the chlorides and nitrates of calcium, strontium, and barium the reaction is of the nature of repulsion alone, as toward the iodides, sodium chloride, etc., given in the table. Toward salts of the heavy metals, as cupric chloride, cupric sulphate, mercuric chloride, zinc chloride, aluminium chloride, the Paramecia are attracted, as toward acids.

From the facts given in the above table and discussion the following conclusions may be deduced: —

1. The base-forming elements of the alkali and earth alkali groups when present in a compound tend to produce the motor reaction in Paramecia whenever the animals come in contact with these compounds, even in very dilute solutions. This may be put in terms of the modern theory as follows: the kations of the alkali and earth alkali groups have a strong tendency to induce the motor reaction in Paramecia. As a consequence these infusoria are kept from entering such solutions even when the solutions are very weak.

2. The ions of the acid-forming elements, such as the halogens, and in fact the anions of *all* acids, do not themselves tend to cause the motor reaction in Paramecia, except when present in such strength as to be immediately destructive. On the contrary, they tend to throw the Paramecia into such a physiological condition that contact with any solution containing no anions produces the motor reaction. As a consequence, Paramecia tend to congregate in regions where it is mainly the anions that give character to the solution.

The fact that the same result as for acids is obtained for salts in which the anion is much the most powerful component, as in potassium fluoride, shows of course that the results with acids are not due to the hydrogen ion of the acids.

3. Compounds that contain both kations of the alkali elements, and anions, may give a combination of these results, — a tendency to cause the motor reaction (with its reversal of cilia) both when the animals enter the solution (due to contact with the kations), and when they leave the solution (effect of the anions). In this case the Paramecia collect in a ring about a drop of the compound.

4. Salts that contain the relatively inactive kations of one of the heavy metals, as aluminium, copper, zinc, mercury, produce an effect due only to the anion, hence their effect is like that of an acid.

These conclusions in regard to the different effects of the anions and kations on the motor reactions of Paramecium certainly seem to invite a discussion of the relation of the activities of the ions during the passage of an electric current through a solution to the movements of the Paramecia under the same conditions. As is well known, when a continued current is passed through the water containing Paramecia, they all swim with one accord to the kathode, — thus in the same direction as that in which the metallic ions are moving. It is difficult to resist the impression that there must be some such relation between the movements of these ions and the movements of the organisms as has been suggested by Loeb and Budgett.[1] Yet a discussion of this matter will hardly lead to valuable results until it can be accompanied by a careful re-examination of the phenomena of electrotaxis, with especial reference to the relation of the electrotactic movements to the general motor reaction of Paramecium described in the second of these Studies. We possess already a detailed account of the movements of the cilia in electrotaxis, due to Ludloff,[2] but these observations, as well as my own observations on electrotaxis, published in I, were made before the general plan of the motor reactions of Paramecium was known, so that a re-examination might lead to the discovery of facts of fundamental significance.

4. RELATIVE REPELLENT POWER OF RELATED SUBSTANCES.

In the first part of this paper I have shown that with relation to their action upon Paramecium, chemical substances are divisible into two classes, in one of which the repellent power is very great in proportion to the injurious effects, while in the other class such slight repellent power as exists is due directly to injuries caused. In this latter class a quantitative study of the relative repellent powers of the different members shows of course that the power of setting in operation the motor reaction of Paramecium is in a general way proportional to the injuriousness of the solutions. But to what factor are the relative repellent powers among themselves in the members of the *first* group due? I have already shown that the relative repellent power of members of the first group, as compared with that of members of the second group, is due to the presence in preponderating amounts of some metal of the alkali or earth alkali series. But this leaves untouched the question of the relative repellent powers of members of the first group among themselves. For example, will sodium chloride be more or less repellent than potassium chloride, and why?

On the question of the relative repellent powers of different members of this group much labor was spent, with rather unsatisfactory results. This is due to the fact that the different compounds have specifically different ways of affecting the Paramecia, some modifying the general motor reaction in one way, some in another, — although never changing the essential character of the reaction. This is a matter worthy of special study. Chemotaxis is a complex phenomenon, the motion to or from a substance being the indirect resultant of the specific physiological effects of this substance upon the different phases of the motor reaction. These specific physiological effects are therefore the primary phenomena. This fact was not recognized at the time my work was done, so that full records were not made on these points. On a few substances observations were made, which I will give here as examples. As shown in II, the typical motor reaction is as follows: (1) the cilia are reversed, causing the Paramecia to swim backward; (2) the aboral cilia of the anterior half of the body strike transversely toward the oral side, turning the animal toward the aboral side; (3) the cilia all strike backward as at first, carrying the animal forward over a path which lies at an angle to the original path. This reaction is given in characteristic form when the animal comes in contact with a weak alkali; all the parts are fairly though not excessively pronounced. On coming in contact with a strong acid solution, the Paramecia dart furiously backward a long distance. Then they turn and dart furiously forward, so that they often thus rush directly into the drop of acid, and perish. If their course after turning, however, tends to carry them away from the acid, when they strike the water into which no acid has diffused they react again by reversing and turning, — showing that the acid has put them into a peculiar physiological condition. Some acid substances cause the reaction with certain of its typical features nearly or quite suppressed. Thus when the animals swimming forward come in contact with diffusing mercuric chloride, they dart straight backward, the anterior end being curved a little to one side; at the same time the body begins to swell. Then they start straight forward again, the turning usually being nearly or quite omitted. Paramecia which come in contact with a drop of $\frac{2}{5}$ per cent calcium chloride solution while swimming forward, pass in without hesitation, but are excited and the forward course is hurried, so that they soon pass out. They thus never remain quiet in the calcium chloride, and as a result it remains nearly empty, the Paramecia gradually settling down elsewhere. Yet one cannot say that the calcium chloride repels the animals; it merely excites them, without causing even a change in the direction of motion. Paramecia introduced directly into 2 per cent barium nitrate solution are paralyzed instantly. But in three or four minutes they revive, and swim slowly about. In 2 per cent calcium nitrate solution, on the other hand, they are at once violently excited, swimming forward and backward swiftly. In solution of strontium nitrate they are likewise excited, but much less so. In 1 per cent sodium bromide and sodium iodide they first dart backward, then turn, then swim forward, then backward again, — repeating the entire typical motor reaction many times. By 1 per cent potassium iodide each part of the motor reaction is almost indefinitely prolonged; they swim backward perhaps ten minutes at a time, then they turn toward the aboral side, and *keep on turning*, whirling thus on the short axis for twenty minutes or more. In 10 per cent solution of cane sugar they at first give no reaction; gradually as plasmolysis occurs they begin to swim backward, then turn, then swim forward, repeating this, as in sodium bromide solution. It will readily be seen in view of these peculiarities in reaction, that it is not always possible to classify the conduct of the Paramecia at once into the two categories of attraction and repulsion.

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[1] LOEB and BUDGETT: Archiv f. d. ges. Physiol., 1897, lxv, p. 518.

[2] LUDLOFF: *Ibid.*, 1895, lix, p. 525.

Moreover, toward many compounds there exist great individual differences among the Paramecia as to the reaction given; at times a few individuals will swim directly into a solution, while the rest leave it empty. There is thus no absolute criterion for determining at what strength the repulsion of a solution really ceases. Moreover, the sensitiveness of the Paramecia to solutions of different strength of a given substance seems to vary according to something like Weber's law, — so that the least observable differences in reaction are between that due to a solution of given strength, and that due to a solution of twice this strength. It is often difficult to determine, for example, whether the repellent power of a substance ceases at $\frac{1}{40}$ or $\frac{1}{80}$ per cent; but upon this decision may depend our conclusion as to which of two substances shows the greater repellent power.

The following results upon certain salts are, however, based upon a very large number of experiments, and so far as they go, may be given with confidence. The method of experiment was that already described, the two substances being compared always under exactly similar conditions in all respects, by introducing a drop of each beneath the cover-glass of the same slide of Paramecia. A special study was made in this way of the halogen salts of the alkali metals, — the chlorides, bromides, and iodides of lithium, sodium, potassium, rubidium, and ammonium, together with the chloride of cæsium. This study was made at Hanover, and therefore upon Paramecia that were repelled by all these salts, so that the repulsion was not confused with any accompanying attraction.

As explained in connection with certain similar results given in I, it is not possible to give a figure which shall represent in a general way the weakest concentration of any one of these substances that causes repulsion in the Paramecia, on account of the very great variation in the reaction of Paramecia from different cultures and under different conditions. For example, on testing Paramecia from two different cultures at the same time, it was found that those from one culture were decidedly sensitive to lithium chloride in a $\frac{1}{40}$ per cent solution, leaving a drop of this strength empty for a long time, while those from the other culture were quite indifferent to the same chemical even in a strength sixteen times as great, swimming in and out of a drop of $\frac{2}{5}$ per cent solution of lithium chloride without reaction. But the *relative* sensitiveness of Paramecia to two or more different chemicals seems to remain fairly constant, so that the order of sensitiveness to different chemicals can be determined. I give therefore the order of sensitiveness to the different metal compounds of each halogen, — the test of sensitiveness being the relative strength of solution of the different substances that causes repulsion.

For the chlorides the order is as follows, beginning with the most repellent: LiCl, KCl, NaCl, RuCl, CsCl. That is, the repellent power decreases as the atomic weight of the metal component increases, except that the sodium compound is moved down one place, being less repellent than the compound of the heavier potassium. The weakest repellent solution varies from about $\frac{1}{40}$ per cent in the lithium chloride to about $\frac{1}{2}$ per cent in the chloride of cæsium.

For the bromides the order is, beginning with the most repellent, LiBr, KBr, NaBr, RuBr; for the iodides LiI, KI, NaI, RuI. In these two sets of salts the order is thus the same as for the chlorides. In general, therefore, the repellent powers of the halogen salts of the alkali metals varies inversely with the atomic weight of the metal components, except that in each case the sodium compound is less repellent than the heavier potassium compound. The ammonium salts were found in each case to possess almost exactly the same repellent power as the corresponding salts of potassium.

Neglecting for the moment the inverted order in the potassium and sodium salts, two causes suggest themselves for the inverse proportion between the repellent power and atomic weights of the elements. The halogen component remaining the same throughout a given series, the molecular weights of the salts of course increase with the atomic weights of the metal components. Hence the less repellent compounds have the greater molecular weight, so that a solution of given strength by weight of one of these salts contains fewer molecules than does the corresponding solution of one of the lighter salts. Supposing the molecules to have throughout the same repellent power, the solution of the lighter salt, containing more molecules, will of course be more repellent. A second cause may be the usual greater chemical activity of lighter elements. If both these causes are efficient factors, they of course reinforce each other in the series above given. The reason for the anomalous position of the sodium salts, as weaker than the potassium salts, does not appear.

On comparing the series of the different halogen salts of a given metal, as NaCl, NaBr, NaI, I have found it impossible, in spite of much labor directed upon this point, to determine satisfactorily the relative repellent powers. Apparently the three salts have almost exactly equal repellent powers, and many sets of experiments upon the corresponding series for each of the four metals, lithium, sodium, potassium, and rubidium, have not made it possible to differentiate them. In every case the repulsion was very nearly equal for all, and the slight differences observed in one set of experiments did not hold for the next. It is evident that the two factors given above as possibly reinforcing each other in causing the greater repellent power in the lighter salts of the metal series of a single halogen, would, in the halogen series of a single metal, work in opposite directions. The greater number of molecules in the lighter compounds (as of chlorine) would tend to produce greater repulsion. But the greater chemical activity of the lighter chlorine component would tend to neutralize the effect of the metal component, to which alone the repulsion is due. Possibly it is to this interference that the approximately equal repellent power of the chlorides, bromides, and iodides of a given alkali metal is due.

Much time was spent on a similar quantitative study of the nitrates of the alkali metals and of the earth alkali metals, but without clearly defined results. The different nitrates have specifically different effects on the Paramecia; the repellent power is the resultant of several factors, any one of which may vary independently of the others. Thus the phenomena differ in the different salts, and there is no certain criterion for comparing the repellent powers of two salts having different specific physiological effects.

The above somewhat fragmentary and partially negative results of quantitative studies are given, not only because the data obtained are of themselves of some value, but also because it is of importance to show which lines of investigation promise to be fruitful and which do not. This method of study has thrown chiefly a negative light upon chemotaxis, — showing what it is *not*. The evidence that chemotaxis is not a direct simple phenomenon, due to simple forces of attraction and repulsion, is in itself of importance.

The foregoing study of laws of chemotaxis in Paramecium, while it is believed to contain some results that are new and significant, is not put forth as in any sense exhaustive. Certain laws came forth clearly, while certain lines of investigation proved comparatively unfruitful; these I have set forth for the positive value of the one and the negative value of the other. The work has been done chiefly on inorganic compounds; almost the whole field of organic compounds remains to be examined. Certain facts in the reactions toward inorganic substances remain at present isolated, not falling under any of the general laws above given; thus certain experiments seem to show that there is a strong repulsion exhibited toward iodine, bromine, and chlorine when uncombined, — though in the ionic condition these elements are purely attractive. The effect of metals other than the alkalies and alkaline earths, when uncombined with a strong acid, is of interest, and has not been determined. Finally, an examination of the specific physiological effects of chemical compounds on the different parts of the motor reaction, as well as on the substance and the general functions of the animals, would be of fundamental value. Such a study would not be directed purely upon one aspect, as upon repulsion and attraction, which are really the resultant of complex factors, but should have in view any change produced by the substances studied

SUMMARY AND CONCLUSIONS.

In the introduction to the first of the Studies of which this is the fourth, the program for work was given as the investigation of the life activities of a single unicellular organism as completely as possible, in the hope that when such a study should have been carried to some degree of completeness, it should furnish a key to the understanding of the activities of other organisms, and should throw light on the essential nature of chemotaxis and similar phenomena. The investigation of the activities of Paramecium thus begun is far from being complete, yet I believe it may be said that the results thus far gained have already justified the plan of work. The mechanism of attractions and repulsions has been made out, and the third paper of the series shows that this furnishes at once a key to the understanding of the activities of certain other infusoria (Spirostomum and Stentor). Studies now in progress show that it throws equal light upon the activities of other, more distantly related, organisms. All the Studies thus far published have dealt more or less with chemotaxis, and in concluding the present paper upon that subject, it will be well to summarize in the form of brief statements the important conclusions in regard to the phenomena which have gone under this name, drawing upon the previous Studies as well as the present one for this purpose and emphasizing some important points which have not been emphasized elsewhere.

1. The motor reaction by means of which chemotaxis is brought about is identical with that produced by mechanical shock or any other stimulus, so that chemotaxis is not an activity differing *in kind* from others. This motor reaction is as follows: the animal swims backward, turns toward one side which is structurally defined (the aboral in Paramecium), then swims forward.

2. This motor reaction is the same, whatever the position of the chemical or other stimulus, or the part of the body of the animal that it acts upon, so that the same chemical which when acting on the anterior end of the animal causes it to move away, causes it when acting upon the posterior end to approach. The direction of motion has no relation to the localization of the stimulus. The usual relation of the direction of motion to the position of the stimulus depends upon the fact that owing to the forward motion, stimulation almost universally occurs at the anterior end.

3. Negative chemotaxis or repulsion toward any substance is due merely to the fact that this substance sets in operation the one motor reaction above described. The Paramecium then does not come to rest until the random movements thus caused have carried it by chance, as they must in time, into a region containing none of the substance which causes the motor reaction.

4. Different chemicals have different specific effects upon the various component parts of the above reaction; one part may be intensified or weakened, while other parts are modified in some different way. The motion to or from the chemical is the resultant of these specific effects taken together. The direction of motion, known as positive or negative chemotaxis, is therefore a complex phenomenon, resulting indirectly from various factors.

5. Paramecia are nearest the quiet condition when in a weakly acid solution. Paramecia at rest under normal conditions are in such

a weakly acid solution, due to the carbon dioxide in the water, produced by themselves.

6. When Paramecia in such a weakly acid solution come in contact with a solution containing no acid, the motor reaction (with its reversal of cilia) is produced, so that the Paramecia do not pass out of the acid solution.

7. On passing from a neutral solution to a weakly acid solution no reaction is caused, but if now the Paramecium attempts to pass back again out of the acid solution, the reaction is produced (as in 6), so that the animal remains in the acid solution. The acid thus changes the physiological condition of the Paramecium, so that it now reacts to the neutral fluid.

8. The anions or acid ions present in salts tend to throw the Paramecia into the same physiological condition as the pure acid, so that the Paramecia afterward react when they come in contact with a fluid not containing these ions.

9. It results from 5, 6, 7, and 8 that Paramecia collect in solutions having a weakly acid reaction, or in solutions of salts in which the anion is especially active. Thus the so-called positive chemotaxis of Paramecium is brought about. The positive chemotaxis toward certain nutritive fluids, such as meat extract, is due to the acid reaction of the latter, not to its nutritive value.

10. Alkaline solutions and solutions of salts containing the kations of the alkali metals or of the earth alkali metals produce in a marked degree the motor reactions of Paramecium. As a result the Paramecia do not come to rest until their random movements have brought them into a solution which does not contain a large proportion of these ions. Thus the apparently negative chemotaxis of Paramecia toward these substances is brought about. In a series of salts of any one of the halogens with the different alkali metals, the power of producing the motor reaction (and hence the repellent power) decreases as the atomic weight increases, *except* that potassium salts are throughout more repellent than the lighter sodium salts.

11. In solutions of salts containing the kation of a strong alkali metal, as well as a powerful anion, as of fluorine or chlorine, both of the ions may act on the Paramecia, — the former causing the motor reaction when the Paramecium attempts to enter the solution, the latter when it attempts to leave it. In this case the Paramecia gather in a ring in the outer margin of a drop of the solution and are apparently both repelled and attracted by it.

12. The chief factor causing the motor reaction which results in negative chemotaxis is not the injuriousness of the substance, but is of a chemical nature. Two substances of equally injurious properties have by no means equal repellent powers, but those which are alkaline or contain kations of the alkali or earth alkali elements are much more repellent than most others, without regard to injurious effects.

13. Nevertheless, any substance which directly and severely injures the Paramecia does thereby cause a motor reaction. But this reaction is not produced till the injury has occurred and it is too late to save the Paramecia. They therefore swarm into chemical substances of certain sorts by which they are immediately killed.

14. It is therefore possible to distinguish (1) reactions due purely to the chemical nature of the substance, the reacting Paramecia being entirely unharmed, and (2) reactions due purely to the injury produced by the substance, without regard to its chemical nature. Reactions of the latter class are not sufficiently precise to be protective.

The investigation of which the results are presented in the foregoing paper was pursued in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes, at Put-in-Bay, Ohio, in the summer of 1898, and continued at Dartmouth College during the following autumn. I desire to express my sincere thanks to the officials of the U. S. Fish Commission and to Professor J. E. Reighard, Director of the Survey, for courtesy and assistance in every way during the progress of the work.

THE CHEMISTRY OF THE MELANINS.

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INTRODUCTORY.

THE name "melanin" is a generic term which is used to include all of the dark brown or black animal pigments, whether formed in the body by normal processes, or under pathological conditions. Owing in a measure to the great interest which these substances possess for the pathologist they have been the subject of a number of researches of a chemical nature, yet for some reason these researches have been so fruitless that at the present time we are not in a position even to define a melanin in a chemical sense; in fact, we are not all agreed as to what chemical elements are necessary constituents of a melanin molecule. If we consider for a moment the methods which have been employed for the isolation and purification of the pigments, and at the same time grant that these substances may be very sensitive to the action of chemical reagents, and that it is also within the bounds of possibility that the composition of the pigment, like that of hæmoglobin, is different for different animal species, we will then be in a position to appreciate just such a discordance of analytical results as that which actually exists.

Since these substances have been looked upon as very stable organic compounds, the general method which has been employed for their isolation from the tissues consists in submitting the pigmented material to such chemical processes as will decompose or remove adherent impurities, on the assumption that the stable pigment will remain unaltered by the reagents that have been used. This has been variously accomplished by the action of acids or alkalies, by artificial gastric digestion,[1] and by putrefaction.[2] The residual pigmented material was formerly submitted to various purifying processes, or analyzed without further purification in accordance with the experimenter's attitude towards the prevailing view that the pigments are derived from the non-proteid part of the red coloring matter of the blood, and the consequent expectation of showing the presence or absence of iron in the product obtained.

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[1] BRANDL and PFEIFFER: Zeitschrift für Biologie, 1890, xxvi, p. 348.

[2] DRESSLER: Prager Vierteljahrschrift, 1866, iv, p. 9. (Quoted from Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 79.)

Three years ago Abel and Davis[1] showed that the coloring matter of the negro's skin and hair consists of a colorless ground substance which is stained with the pigment, and that in order to remove the pigment from this ground substance a certain definite order of chemical procedure is absolutely necessary. Since these conditions have not been met by most experimenters it seems highly probable that many of the pigments hitherto described are not true pigments, but so-called granules, and that the supposed solutions of pigments in alkalies were only suspensions in such a fine state of division as to pass easily through filter papers. In fact, one who is acquainted with pseudo-solutions of pigmented granules can now easily understand from the descriptions in the literature that many of these so-called solutions of pigment were not solutions at all.

This discovery, however, does not bring about the desired conformity in analytical results, for Abel and Davis found that the composition of their true pigments is easily subject to change by subsequent treatment of the material with alkalies. Schmiedeberg,[2] also, who was perfectly familiar with the distinction between granules and true pigment, found that the pigment acid obtained by two slightly different processes from a melanotic sarcoma differed so widely in chemical composition as to be represented by such fundamental formulas as these,

α-Sarco-melaninic acid, $C_{68}H_{65}N_{11}SO_{26} \cdot 10\frac{1}{2}\ H_2O$.
β- " " $C_{68}H_{67}N_{18}SO_{26} \cdot 3\frac{1}{2}\ H_2O$.

In fact, it seems that the material called a melaninic acid is even more sensitive to treatment with chemical reagents than the pigmented granules themselves. For this variation in the composition of a given melanin two causes may be assigned.

First. The material called a melanin may be a mechanical mixture of the pigment with some impurity, the latter being gradually removed by the action of chemical reagents.

Second. The melanin may be a substance whose molecule is very sensitive, and easily changes its composition in the process of chemical manipulation.

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[1] ABEL and DAVIS: Journal of experimental medicine, 1896, i, p. 381.

[2] SCHMIEDEBERG: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 77.

On either assumption it would be interesting to know how far this change in composition can proceed, and for the purpose of investigating this question the work which is about to be described was undertaken.

PREPARATION OF MELANIN GRANULES.

Black hair from the tail of the horse having been carefully sorted and cleansed by washing with water, was closely packed in glass jars and then treated with concentrated hydrochloric acid[1] in great excess. From time to time the contents of the jars were stirred, and as the material became disintegrated successive additions were made until 450 grams of hair had been used for every litre of acid. After the final addition of hair the contents of the jars were allowed to stand, with occasional stirring, during the period included between August 1 and October 7. After the acid had thus acted for ten weeks at the summer temperature the hair was found to be completely disintegrated, the product consisting of a dark brown liquid beneath which was a muddy gelatinous mass, there being no sharp line of demarcation between the two. This material was treated with an equal volume of tap-water, and as subsidence had occurred fairly well after standing over night, the muddy liquid was siphoned from the black sediment. A large volume of tap-water was then added and the vessel thoroughly shaken. As subsidence did not occur after several days, enough caustic soda was added to give the liquid a decided alkaline reaction, and the material was then made faintly acid with hydrochloric acid. Under these conditions the granules settle immediately, leaving a perfectly colorless liquid which can be sharply siphoned off. A large volume of tap-water was again added, and this process of washing by decantation was repeated many times, the alkaline fluid being occasionally filtered through coarse filter-paper, and the last washings made with distilled water instead of tap-water.

The soluble proteids being thus removed, alcohol was substituted for water and the washing by decantation continued, the alcohol being made alternately alkaline and acid, as in the case of washing with water. After the last portion of alcohol had been siphoned off, the muddy mass of granules was placed on filters and washed successively with absolute alcohol and ether. Experience has shown that it is not advisable to use a filter-pump for this or any filtration which will be mentioned in this paper. The liquid will run continuously for many hours, through a plain filter, and while the operation seems quite slow at the beginning, it does not become any slower as the filtration proceeds. In case an analysis of the granules is required, material can be taken with a spatula from the centre of the filter without fear of contamination by fibres of paper.

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[1] ABEL and DAVIS: *loc. cit.*, p. 369.

When a portion of the ether washings left only a slight residue on evaporation, the filters were allowed to stand uncovered until most of the ether had evaporated and then placed in desiccators provided with sulphuric acid and shavings of paraffin. When the material on the filters begins to show cracks it can be removed easily, but after it becomes perfectly dry there is danger of removing filter fibres. The thick paste of granules was ground to a powder in an agate mortar and thoroughly dried in a desiccator over sulphuric acid.

A small weighed portion of this material was heated to constancy of weight at 110° C. From the numbers thus obtained with the known weight of desiccator dry granules obtained from a certain weight of hair, it was found that the dry granules constitute about 5½ per cent of the hair in its normal condition. In the case of the negro's hair Abel and Davis found the proportion of granules to be less than 2 per cent.

PREPARATION OF MELANINIC ACID FROM MELANIN GRANULES.

One hundred grams of caustic potash were melted in a nickel bowl with 10 c.c. of water, and 10 grams of granules were added in small successive portions, the material being continually stirred and a cubic centimetre of water being added from time to time. Immediately after the addition of a portion of granules there was considerable smoking with a decided odor of burnt hair, but the material very soon came into a state of calm fusion. After the last addition of granules, heating was continued for about ten minutes at temperatures which were near 350° C., and the melted mass was finally poured into cold water and allowed to stand in a porcelain bowl until the solution was perfectly cool. The fluid, which possessed a most intense indol odor, was diluted with water to a volume of 1000 c.c. and filtered through hardened filters. On diluting a small portion of the black filtrate with water there was obtained a perfectly transparent Bordeaux red liquid which was immediately recognized as a true solution. The alkaline filtrate was made faintly acid with hydrochloric acid, and after standing twenty-four hours the pale yellow liquid was siphoned from the melaninic acid that had fallen to the bottom. The latter was thrown on filters, washed with hot water for several hours, and spurted from the filters into tall glass jars by means of a jet of hot water. The material was then alternately dissolved in large volumes of extremely dilute alkali, and precipitated by the addition of a trace of hydrochloric acid, the last alkaline solution having been filtered to remove particles of dust and fibres of filter-paper. When alkali is added the melaninic acid goes sharply into solution, and on acidifying such a solution with hydrochloric acid the pigment falls out as a reddish brown flocculent precipitate which settles immediately, leaving a perfectly colorless liquid above. A check test was made to show that ten times the approximate percentage of sodium chloride present could not be precipitated by the addition of any amount of absolute alcohol. As this test gave the desired result, the melaninic acid was washed by decantation with absolute alcohol until the water was removed, when ether was substituted and the washing by decantation continued. The subsequent treatment of the material was the same as that described above for the purification of the granules, except that a large quantity of the melaninic acid was removed from the centre of the filters, ground dry of ether in an agate mortar, and reserved for analysis.

The product thus obtained is a dark brown amorphous powder with a yellowish brown streak which causes the substance to appear lighter in color when it is ground in a mortar. It is insoluble in alcohol, ether, benzene, acetic ether, and chloroform, but quite easily soluble in distilled water, giving to the latter a decided acid reaction to litmus. From its solution in water it is precipitated by dilute hydrochloric or sulphuric acid, but not by acetic acid. When dried for a long time in a desiccator over sulphuric acid the pigment loses its solubility in water, but is soluble in water that contains a trace of sodium hydroxide, forming a fine, transparent red solution. When such a solution is diluted and made acid, a light yellow turbidity occurs, which gradually turns darker and finally collects into reddish brown flocks, leaving a perfectly transparent liquid. The alkaline solution is easily decolorized by passing in chlorine or by boiling with potassium permanganate in proper quantity. When dried at 110° the pigment becomes very difficultly soluble in alkalies. On heating in a tube over a free flame the melaninic acid develops a very offensive indol odor, a colorless deposit occurs on the colder part of the tube, and vapors are given off which yield an intense pyrrol reaction with a pine splinter that has been moistened with concentrated hydrochloric acid. There is, however, no odor of burnt hair produced.

THE COMPOSITION OF MELANINIC ACID.

A quantity of material which was found on microscopic examination to be free from fibres of filter-paper was dried in small portions to a constancy of weight at 110° and analyzed.

In a silver crucible was fused 0.2883 gram of the substance with caustic potash and potassium nitrate for the purpose of making a quantitative determination of sulphur. The cake was dissolved in water, the solution made acid with hydrochloric acid and filtered from a trace of silver chloride. On heating the filtrate and adding barium chloride not the slightest clouding occurred in the course of half an hour, yet the addition of a trace of sulphuric acid produced an immediate precipitate.

I. 0.1823 gram substance gave 0.0008 gram ash = 0.44 per cent.
II. 0.2801 gram substance gave 31.5 c.c. moist nitrogen at 15.5° C. and under a barometric pressure of 762 mm.
III. 0.1856 gram substance gave 0.3939 gram CO_2 and 0.0586 gram H_2O.

Part of the same material, before treating with alcohol and ether, was precipitated additionally six times from a dilute alkaline solution with hydrochloric acid. The alkaline solutions were left freely exposed to the air, and the final product, dried to a constancy of weight at 110°, gave the following analytical results: —

IV. 0.2001 gram substance gave 0.0009 gram ash = 0.45 per cent.
V. 0.2355 gram substance gave 27 c.c. moist nitrogen at 22.5° C. and under a barometric pressure of 763 mm.
VI. 0.1861 gram substance gave 0.3937 gram CO_2 and 0.0568 gram H_2O.

	Percentage composition of ash-free substance.				*Theoretical for*
	II	III	V	VI	$C_{52}H_{36}N_{10}O_{17}$
C	....	58.14	....	57.94	58.20
H	....	3.52	....	3.41	3.36
N	13.18	....	13.07	....	13.06

While the properties of this substance which have been noted are at once recognized as characteristics of the melaninic acids, it might nevertheless be objected that the rather rough treatment to which the material has been subjected is well calculated to so alter its chemical constitution that it bears only a remote chemical resemblance to the melanin that actually exists in the body. As so little is known, however, of the chemical relation between living and dead matter, it seems difficult to decide what chemical processes are to be regarded as permissible for the isolation of a substance from the tissues. The objection offered may therefore with equal propriety be applied to almost every preparation known to physiological chemistry, for what is regarded as very mild treatment to some substances would be most severe when applied to others. It being universally granted that a chemical change is always accompanied by a change of properties, it would seem that we are more concerned with the characters of the substance in question than with the methods by which it has been obtained.

While we believe that the pigment is not identical with that originally present in horse hair we fail to see any sufficient evidence for regarding the former as a product of deep-seated chemical action.

Nor does the absence of sulphur from this material constitute any argument against its acceptance as a true melaninic acid, for Sieber[1] obtained from the choroid coat a sulphur-free pigment, having subjected the material to no more severe an operation than boiling for two hours with ten per cent hydrochloric acid. Later, Hirschfeld[2] obtained a similar result by using an acid of a strength less than five per cent and working in the cold, — a precaution which was employed to avoid the production of artificial melanins, which are liable to be formed by the action of stronger acids on adherent proteids.[3] From this point of view one might think of the pigment that has been described in this paper as an artificial choroid pigment. Such, however, is not the case, for Hirschfeld[4] has shown that when the choroid pigment is treated with melted alkalies, a product is obtained which is free from nitrogen.

The literature shows still another instance of a melaninic acid obtained by melting a natural product with alkalies, namely, the hippomelaninic acid of Nencki and Sieber.[5] Although this substance resulted from a longer treatment with alkali than the one which we prepared, two preparations were found to contain 1.39 per cent and 2.6 per cent of sulphur respectively. Moreover, it is distinguished from the melaninic acid of Hirschfeld in its nitrogen content (10.41 per cent). It therefore seems clear that whether these products are to be regarded as chemically altered pigments or not, their marked chemical differences from one another constitute a conclusive proof that the three physiological pigments which they represent must themselves have corresponding differences. This involves the assumption that in at least one instance the pathological pigment (Nencki's hippomelaninic acid) is different from the normal pigment (melaninic acid from horse's tail) in the same animal species.

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[1] SIEBER: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 363.

[2] HIRSCHFELD: Zeitschr. f. physiol. Chemie, 1889, xiii, p. 418.

[3] See SCHMIEDEBERG: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxix, p. 70; also CHITTENDEN and ALBRO: This journal, 1899, ii, p. 291.

[4] HIRSCHFELD: *loc. cit.*, p. 430

[5] NENCKI and SIEBER: Archiv f. exper. Pathol. u. Pharmakol., 1888, xxiv, p. 17.

An extension of this work is now being carried on in this laboratory which will include other natural pigments as well as artificial melanoid bodies formed by the action of acids on various proteïds.

OXIDATION OF MELANINIC ACID. OXYMELANINIC ACID.

(*a*) **Oxidation in an alkaline medium.** — The following preliminary experiments were made with the melaninic acid from the negro's hair, the process employed for the isolation of the pigment being essentially that described above for the preparation of the corresponding substance from the horse's tail.

A quantity of melaninic acid was dissolved in water which contained enough sodium hydroxide to effect an easy solution of the pigment. As this solution was boiled, successive small portions of a faintly alkaline solution of potassium permanganate were added until the clear liquid between the particles of precipitated manganese dioxide hydrate showed by its faint pink color that the permanganate was in slight excess. A sufficient amount of a solution of oxalic acid was then added to reduce the excess of permanganate and dissolve the precipitate. The solution had a pale yellow color due in all probability to the presence of a slight amount of pigment that had become included in the precipitated manganese dioxide hydrate and had thereby escaped oxidation. The oxalic acid and manganese were removed in the ordinary way with barium hydroxide, and the excess of the latter was precipitated by passing carbon dioxide into the hot solution. The filtered liquid was evaporated to dryness on the water-bath and the small quantity of pale yellow residue was examined. Aside from a very unsatisfactory reaction for pyrrol with a pine stick moistened with hydrochloric acid, and a slight gray charring when heated on the foil, no organic matter could be detected. The various precipitates were also examined for organic carbon with negative results.

Similar experiments were also made with larger quantities of material, using ammonium permanganate as the oxidizing agent, and working at temperatures between 0° and 5° C. Even under these conditions the permanganate is almost immediately decolorized, showing that in alkaline solution the pigment is oxidized with the greatest ease. In this instance organic matter could undoubtedly be proved among the products of the reaction, but its amount was so insignificant that its presence is probably referable to undecomposed pigment.

As it is quite possible that volatile substances formed in these oxidations may have escaped in the manipulation, the following experiment was made in such a manner as to preclude the possibility of any volatile acid or basic substance being lost. About two grams of melaninic acid from the horse's tail were dissolved in a considerable excess of sodium hydroxide, and a gentle stream of chlorine gas was passed into the alkaline solution. For the purpose of retaining volatile basic substances, the end of the apparatus was provided with an absorption bulb which contained more than half enough hydrochloric acid to neutralize the sodium hydroxide in which the pigment was dissolved. The pigment becomes rapidly decolorized, and in a few minutes a solution results which is no more highly colored than a solution of chlorine gas in water. This material was divided into two equal portions, and after adding to one of these the hydrochloric acid solution contained in the terminal bulb, both portions were evaporated to dryness on the water-bath and the residues examined. That which resulted from the acid portion was found to contain a large quantity of an ammonium salt, but neither residue gave the slightest evidence of organic carbon. A similar experiment with the melaninic acid from the negro's hair gave the same result. The conclusion, therefore, seems to be justified that these pigments are easily oxidized in alkaline solution, and that in all probability the oxidation is so complete as to give rise to such simple substances as carbon dioxide, water, and ammonia.

(*b*) **Oxidation in an acid medium.** — When a solution of the pigment in water is treated with chlorine, the result is quite different from that obtained by using an alkaline solution. In the earlier stages of the oxidation the pigment is thrown down in consequence of the acidification of the solution; but its color soon begins to depart, and in the course of half an hour is light reddish-yellow. A continuation of chlorine has now but little effect, for it was found impossible in a trial experiment to completely decolorize a gram of pigment by treatment with chlorine for eight hours. Several portions of pigment which were roughly estimated to aggregate ten grams were treated with chlorine in tall cylinders and the reddish-yellow emulsions were united and warmed on the water-bath. The undissolved material soon became flocculent, leaving a clear, almost colorless supernatant liquid. The solution was filtered off and the pigment, which had become somewhat darker, was spurted into a tall cylinder, and alternately dissolved in sodium hydroxide and precipitated with hydrochloric acid. At each step of this procedure the material was found to be darker in color until a product was finally obtained which in its general appearance is indistinguishable from the melaninic acid originally used for the oxidation. The acid solution was finally siphoned off and the pigment washed by decantation with successive large portions of absolute alcohol. Ether was then substituted for alcohol, and the pigment was finally obtained as a dry dark brown powder by a process similar to that described above for the preparation of melaninic acid. The product dried in a desiccator over sulphuric acid is soluble in water but insoluble in organic solvents. When heated it gives off a very strong skatol odor and produces fumes which impart an intense red color to a pine stick that has been moistened with hydrochloric acid. In fact there is nothing connected with its physical properties or chemical reactions which would suggest that the substance is any other than the melaninic acid from which it was prepared. Having determined the absence of chlorine, the substance was dried to a constant weight at 110° and analyzed.

I. 0.2242 gram substance, after fusion with potassium nitrate and potassium hydroxide, gave no precipitate in acid solution with barium chloride.
II. 0.0914 gram substance gave 0.0006 gram ash = 0.66 per cent.
III. 0.2092 gram substance gave 0.3625 gram CO_2 and 0.0474 gram H_2O.
IV. 0.1770 gram substance gave 0.0382 gram H_2O.
V. 0.1796 gram substance gave 24.1 c.c. of moist nitrogen at 18.5° C. and under an atmospheric pressure of 759.5 mm.

	Percentage composition of the ash-free substance.			*Theoretical for*
	III	IV	V	$C_{18}H_{11}N_5O_{10}$
C	47.60	....	....	47.26
H	2.53	2.42	....	2.41
N	....	....	15.56	15.32

The formation of this substance, which may be called oxymelaninic acid, must therefore be expressed by the following equation :

$$C_{52}H_{36}N_{10}O_{17} + 42\ O = 2\ C_{18}H_{11}N_5O_{10} + 16\ CO_2 + 7\ H_2O.$$

Attention was next given to the soluble products formed by the action of chlorine on melaninic acid in acid solution. The liquid containing these substances was light yellow in color, but became gradually darker as evaporation on the water-bath continued, reaching finally a very dark red and depositing flocks of a brown material which is in all probability identical with oxymelaninic acid. The precipitate was filtered off and a drop of the filtrate was made alkaline with sodium hydroxide. A volatile base was given off which fumed in the air, changed red litmus, and gave dense white vapors with a drop of dilute hydrochloric acid held upon a stirring rod. This base has a very characteristic odor, which strongly resembles that of semen, but the odor of ammonia could not be detected. Suspecting the base to be a substance closely related to if not identical with putrescine, a large portion of the solution was made alkaline with sodium hydroxide and distilled, the distillate being received in a small quantity of dilute hydrochloric acid. The acid distillate was evaporated on the water-bath, platinum chloride was added, and the precipitated platinum compound was recrystallized from hot water and analyzed.

I. 0.2177 gram substance gave 0.0923 gram platinum.
II. 0.2365 gram substance gave 0.1027 gram platinum.

	Theoretical for $(NH_4)_2PtCl_6$	*Found.* I	II
Pt	43.79	43.60	43.43

So large a quantity of this substance (which is undoubtedly ammonium platinum chloride) was obtained that we must look upon its precursor as the principal nitrogenous product of the oxidation. An analysis of the salt as it was precipitated by platinum chloride without crystallization from hot water gave 39.89 per cent of platinum. This would indicate that with the ammonium platinum chloride there is also precipitated a more soluble platinum compound which contains less platinum than does ammonium platinum chloride.

A comparatively large quantity of the original acid solution was made alkaline with sodium hydroxide and allowed to stand over night under a bell-jar with a small quantity of very dilute sulphuric acid. On removing the bell-jar there was a most pronounced odor of ammonia, although no such odor could be detected immediately after the solution had been made alkaline. The drop of sulphuric acid was diluted with a little water and various alkaloidal reagents applied, but with negative results. Unsuccessful attempts were also made to shake out the base from an alkaline solution with chloroform and ether.

Part of the original acid solution was treated with benzoyl chloride and sodium hydroxide. The liquid was shaken out with ether, and after the ether had been evaporated the residue was boiled with hydrochloric acid. The benzoyl compound is very resistant but finally goes into solution. On making this solution alkaline with sodium hydroxide an odor like that of putrescine can be detected. If this substance is related to putrescine it is probably one of its numerous isomers, which is far more sensitive to the action of alkalies than putrescine itself. Although the base is formed in relatively large quantity by the action of chlorine in acid media on the melaninic acids from the horse's tail, from human melanotic sarcoma, and from the negro's hair, no method has yet been found for its isolation from the products of the reaction. We believe that the substance can be identified by an analysis of its benzoyl compound, but it is apparent from what has already been stated that to obtain the base itself some very special method of manipulation must be used.

CONCLUSIONS.

(1) An easy and convenient method for the preparation of the pigmented granules in any desired quantity from hair consists in treating sorted hair with concentrated hydrochloric acid for a long time at ordinary temperatures.

(2) It is possible to prepare from these granules two substances that must be regarded as melaninic acids, namely, melaninic acid and oxymelaninic acid. Both of these substances are free from sulphur, and in this regard they do not differ from the pigment which both Sieber and Hirschfeld isolated from the choroid coat. One of these melaninic acids was shown not to change its composition by exposure of its solution in dilute alkalies to the oxidizing action of the air, and the other was prepared in such a manner as to suggest that it is not likely to undergo chemical change without deep-seated decomposition. The relation in composition between these two melaninic acids is expressed by the following equation: —

$$C_{52}H_{36}N_{10}O_{17} + 42\ O = 2\ C_{18}H_{11}N_5O_{10} + 16\ CO_2 + 7\ H_2O.$$

This reaction, which occurs under the influence of chlorine gas, strongly reminds us of the oxidation under similar circumstances of various organic compounds which contain several aromatic nuclei in combination, and which lose in the oxidation an approximately equal number of carbon and hydrogen atoms. The oxidation of naphthalene by means of chlorine to form phthalic acid may serve as an instance.

$$C_{10}H_8 + 4\ O = C_8H_6O_2 + 2\ CO_2 + H_2O.$$

It is of interest to note the relation in chemical composition between the various pigments that have been obtained from the horse's tail. For this purpose the following formulas have been constructed: —

1. $C_{58}H_{68}N_{10}O_{16}$.
2. $C_{58}H_{50}N_{10}O_{18}$.
3. $C_{62}H_{83}N_{10}O_{17}$.
4. $C_{86}H_{22}N_{10}O_{20}$.

Formulas (1) and (2) represent two analyses of a pigment which was prepared by Nencki and Sieber.[1] From the analytical data given fundamental formulas have been constructed according to Schmiedeberg,[2] and from these formulas that for sulphuretted hydrogen has been subtracted. The expressions thus obtained were divided by such a quantity as would bring the number of nitrogen atoms to ten. The formulas show progressive oxidation as we pass from (1) to (4). The last member of the series contains relatively more oxygen than any melaninic acid hitherto prepared, and has therefore been termed oxymelaninic acid.

(3) The sulphur-free pigments prepared by me are easily and completely oxidized in akaline solution even at temperatures that are very near 0° C.

(4) By treatment of one of these sulphur-free pigments with chlorine in an acid medium, a volatile basic substance is formed which has an odor very similar to that of putrescine, is easily decomposed by treatment with alkalies leading to the formation of a large amount of ammonia, and yields a benzoyl derivative from which the base can be regenerated. The production of this base from melanins forms part of a chain of evidence to show a chemical relation between these pigments and proteids. E. Schulze and Winterstein[3] have obtained ornithin by hydrolysis of arginine with barium hydroxide, and Ellinger[4] has succeeded in isolating putrescine from the putrefactive products of ornithin. As arginine is a common hydrolytic product of all proteids, it follows that in the proteid molecule there exists some grouping of atoms which corresponds to putrescine and which is a necessary part of the proteid molecule. The discovery of a base, therefore, which closely resembles putrescine in its physical and chemical properties, and which is a common decomposition product

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[1] NENCKI and SIEBER: Archiv f. exper. Pathol. u. Pharmakol., 1888, xxiv, p. 20.

[2] SCHMIEDEBERG: *Ibid.*, 1897, xxxix, p. 2.

[3] SCHULZE, E., and WINTERSTEIN: Berichte der deutschen chemischen Gesellschaft. 1897, xxx, p. 2879.

[4] ELLINGER: *Ibid.*, 1899, xxxii, p. 3183.

of three melaninic acids, suggests that some similar grouping exists in the melanin molecule. This conclusion is also in conformity with the results which Nencki[1] obtained by a study of the products of artificial pancreatic digestion. He has shown that the bromine derivative of tryptophan consists of at least two substances, one of which is a brown pigment whose composition (reckoned bromine free) is almost identical with that of hippomelaninic acid.

It is not my purpose to offer in this paper any theory of my own as to the chemical processes by which the normal or the pathological pigments are derived from proteids. Nencki[2] holds that the protein-chromogens formed during pancreatic digestion may well be the mother substance of all the animal pigments. Schmiedeberg,[3] as already stated, has prepared artificial melanins from proteids, and has given an outline in a broad and masterful way of the possible chemical processes involved in the derivation of the natural pigments from their precursors. Very recently Nencki[4] has remarked that Schmiedeberg's artificial melanins are apparently derivatives of proteinchromogen. Chittenden and Albro[5] have recently verified and extended Schmiedeberg's observations, and have shown that antialbumid and hemipeptone can yield artificial melanins whose percentage composition enables a comparison to be made, in this respect at least, with the natural melanins. Much work of an exact chemical nature still remains to be done before we can make any very definite statements as to the manner in which the natural pigments are formed from their proteid precursors.

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[1] NENCKI: *Ibid.*, 1895, xxviii, p. 560.

[2] NENCKI: *loc. cit.*, p. 566.

[3] SCHMIEDEBERG: *loc. cit.*

[4] NENCKI: Footnote to Loew's abstract of Schmiedeberg's paper in Jahresber. ü. d. Fortschr. d. Thier-Chemie, 1896, xxvii, p. 13.

[5] CHITTENDEN and ALBRO: This journal, 1899, ii, p. 291.

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DIRECT AND REFLEX ACCELERATION OF THE MAMMALIAN HEART WITH SOME OBSERVATIONS ON THE RELATIONS OF THE INHIBITORY AND ACCELERATOR NERVES.[1]

By REID HUNT.

[*Associate in Pharmacology, Johns Hopkins University.*]

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[1] These experiments were performed in the Physiological Laboratories of the Johns Hopkins Medical School, and of the College of Physicians and Surgeons, New York; a summary of some of the results obtained was presented at the meeting of the American Physiological Society in December, 1898, and published in the Proceedings of the Society (This journal, 1899, ii, p. ix).

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PART I.

EXPERIMENTS ON THE ACCELERATOR NERVES.

Methods of investigation. — Most of the experiments described in this paper were performed on dogs, although some were made on cats and rabbits; it is to the dog, however, that the conclusions drawn are intended to apply primarily. The animals were always thoroughly anæsthetized in some manner. Curare was also frequently given; in these cases an effort was made to prevent the animal's temperature from falling during the experiment, either by warming the air (which was also saturated with aqueous vapor) or by keeping the animal on a zinc box filled with warm water. If during the course of an experiment the blood pressure became very low, intravenous injections of warm normal saline or of Ringer solution were frequently made; by means of such injections the blood pressure and heart rate could be kept near the normal for a long time, and results of value obtained from experiments which would otherwise have yielded none.

The heart rate and blood pressure were recorded by the mercury manometer in the usual manner; the readings of the blood pressure given have been corrected for the weight of the solution of sodium carbonate used to prevent coagulation of the blood. In some experiments Hürthle's spring manometers, connected either with an artery or with a cannula passed into one of the ventricles, were extensively used.

The accelerators were usually exposed by resecting the first rib and opening the pleural cavities; such a procedure allows of a much more thorough exposure of the stellate ganglia and their branches than does the longer and more difficult method described by Schmiedeberg.

Full details of the individual experiments will be given throughout the paper.

TONIC ACTIVITY OF THE ACCELERATORS.

The views of physiologists seem to differ widely on the question whether the accelerator nerves are in a condition of tonic activity or not. In some of the leading English[1] and German[2] text-books on physiology the statement is made that these nerves are only occasionally, not continually, in a condition of activity. In other text-books both of physiology and of medicine and frequently in the writings of physiologists it is either stated explicitly that these nerves are usually in a condition of activity or the assumption is made that this is the case. Other authors again do not refer to the question at all.

Apparently the basis for the statement that the accelerators are not in a condition of tonic activity is the work of the Cyons.[3] These physiologists, unlike von Bezold, failed to find any decrease in the heart rate in experiments on rabbits as a result of the section of the spinal cord or of the extirpation of the inferior cervical and stellate ganglia. The later investigations of Tschirjew,[4] Stricker and Wagner,[5] and of Timofeew,[6] all agree in showing that these nerves are usually in a condition of tonic activity in rabbits as well as in dogs; moreover E. v. Cyon himself, in a recent article giving the results of experiments performed for the most part upon rabbits, speaks as though he considers these nerves to be in a condition of tonic activity.[1]

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[1] WALLER: Introduction to human physiology, 3d ed., 1891, p. 107.

[2] LANDOIS: Lehrbuch der Physiologie des Menschen, 7th ed., 1891, p. 807.

[3] CYON, M. and E.: Archiv f. Anat., Physiol., u. wiss. Medicin (Reichert and du Bois-Reymond), 1867, p. 406.

[4] TSCHIRJEW: Archiv für Physiologie, 1877, p. 164.

[5] STRICKER and WAGNER: Sitz.-Ber. d. kais. Akad. d. Wiss., 1878, math.-naturw. Cl., 77, Abth. III, p. 111.

[6] TIMOFEEW: Quoted in Centralblatt für Physiologie, 1889, p. 235. Timofeew did not observe any immediate effect upon the heart rate of cutting the accelerators; about three days after the operation, however, slowing of the heart began, and seems to have been permanent. That no immediate slowing of the heart occurred in these experiments may have been due to a reflex diminution of the tonicity of the vagi (which of itself would lead to an acceleration) resulting from

My own experiments, some of which have already been published,[2] lead me to believe that the accelerators must be considered as almost always in a condition of tonic activity, in fact much more constantly so than the cardio-inhibitory nerves.

So far as I know the only question in relation to the tonic activity of the accelerators discussed by previous writers is the effect of the section of these nerves upon the rate of the beat of the ventricle; this as well as some other problems will be discussed below.

Effect of the section of the accelerator nerves upon the heart rate; resistance of the accelerator centres. — I have very little to add to what I have said in my previous paper as to the effect upon the heart rate of cutting the accelerator nerves; in the large number of additional experiments which I have made, I rarely have found a case in which they were not in a condition of tonic activity. This was true whatever the drug used for anæsthesia, and when anæsthesia was produced not by drugs but by section of the crura cerebri or by compression of the cerebrum; also whether the vagi were intact or had been divided.

One criticism which may be made against some of the experiments upon the effect of cutting the accelerators should be referred to here. The operation necessary to expose thoroughly the accelerator nerves sometimes leads to a lowering of the mean blood pressure; since the work of v. Bezold[3] and Tschirjew it has been generally believed that a low blood pressure acts of itself as a stimulus to the centre of

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the operation; I have referred to this point in a previous paper (Journal of experimental medicine, 1897, ii, p. 160).

Hering (Archiv f. d. ges. Physiol., 1895, lx, p. 468) observed an increase in the heart rate to follow extirpation of the stellate ganglia (the vagi being intact); after a few days the rate returned partially to the normal. Subsequent section of the vagi caused but little increase in the heart rate. These results seem also to indicate a tonic activity of the accelerators, the acceleration immediately following the operation probably being due to a diminution of the tonic activity of the vagi.

[1] von Cyon, E.: Archiv f. d. ges. Physiol., 1898, lxx, p. 242.

[2] Hunt: Journal of experimental medicine, 1897, ii, p. 158.

[3] von Bezold: Centralblatt f. d. med. Wissenschaften, 1866, pp. 819, 820.

the accelerator nerves.[1] Unless this factor is taken into consideration the objection may be made that an abnormal stimulation of the accelerators by low blood pressure has been interpreted as tonic activity of the centre. There were however among my experiments many cases in which as a result of careful operation the blood pressure was high (probably very near the normal) when the accelerators were cut, and yet there was a very marked slowing of the heart. The following figures from an experiment upon a rather small dog may serve as an illustration of this point: the blood pressure was 130 mm. of mercury, the heart rate 39½ in 10 seconds; section of the accelerators on the right side caused the blood pressure to fall to 122 mm., while the heart rate decreased to 26½ beats in 10 seconds; section of the accelerators on the left side caused no change in the heart rate, but the blood pressure fell to 95 mm.

On the other hand I have in a few cases obtained results to which the above objection may properly be made. Occasionally an animal was found in which the accelerators seemed to be in a condition of maximum acceleration, that is, electrical stimulation of the nerves after their section did not cause the heart to beat more rapidly than it had been beating before their section; in all such cases the blood pressure was very low. An example of such an experiment will be given below.

Section of the accelerator nerves in my experiments seldom led to a marked lowering of the blood pressure; hence the criticism cannot be made against these experiments which the Cyon brothers made against the work of von Bezold. Von Bezold cut the spinal cord and attributed the slowing of the heart which followed to the cutting off of the accelerator impulses; the Cyons attributed it to the fall of blood pressure resulting from the dilatation of the blood vessels of the abdominal organs when the spinal cord is divided.

The resistance of the accelerator centre to influences which reduce or entirely abolish the irritability of other physiological centres is very marked. Thus, while the tonic activity of the cardio-inhibitory and of the vaso-motor centres is lowered by such drugs as chloral, chloroform, and ether, and by excessive amounts of curare, these drugs seem to have but little effect upon the accelerator centre. In one experiment upon a dog, for example, after a very large amount of curare had been injected into a vein, the blood pressure fell from 83 to 22 mm. of mercury, and section of the vagi and stimulation of their peripheral ends had no effect upon the heart rate; yet section of the accelerator caused the heart rate to decrease from 29 to 23 beats in 10 seconds. In another experiment the blood pressure was 27 mm.; section of the accelerators caused the heart rate to decrease from 36 to 26½ in 10 seconds. The accelerators were also found to be in activity in an animal which had been under the influence of ether for five hours, and in others in which severe operations and great loss of blood had led to an extremely low blood pressure. In a few animals, however, the temperature of which had fallen very low, the accelerators were not found in tonic activity.[1]

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[1] Asp (Sitz.-Ber. d. sächs. Gesellsch. d. Wiss., math.-phys. Cl., 1867, p. 173) and Nawrocki (Beiträge z. Anat. u. Physiol., Festgabe für C. Ludwig, 1874, p. 220), however, consider the acceleration in these cases to be due to a diminution of the tonic activity of the vagi.

Although section of the accelerator nerves has seldom failed to cause a slowing of the heart, yet the extent of this slowing has varied widely in the different experiments; in exceptional cases the rate after their section has been but one half or less than the previous rate. To what extent the variability of the results was due to differences in individual animals and to what extent to other causes it is impossible to state. In the case of the inhibitory fibres to the heart it is not difficult to determine the influences (drugs, etc.) which increase or diminish their tonus, and in the same animal this tonus may vary within wide limits in short periods of time; in fact the cardio-inhibitory centre seems to be in a condition of very unstable equilibrium. The tonus of the accelerator nerves is not so easily affected; I have been unable to find any constant relation between such factors, *e. g.*, as the degree of anæsthesia, the condition of the blood pressure and of the respiration, etc., and the extent of the tonus of the accelerators, nor do I know of any grounds for supposing that this tonus undergoes changes comparable to those of the cardio-inhibitory centre. There are, moreover, indications that the condition of the heart itself plays a very important part in determining the effect of cutting the accelerators; it is even probable that changes in this organ are of more weight in this connection than changes in the accelerator centre.

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[1] The resistance of the peripheral endings of the accelerators is also remarkable. Thus stimulation of the spinal cord in the cervical region has caused a marked acceleration of the heart, although, as a result of excessively large doses of curare, the blood pressure was not affected. In the last stages of asphyxia, also, stimulation of the accelerators is effective, as has already been described by Bowditch. Even when the heart is dying and the ventricles have ceased to beat, stimulation of the accelerators will sometimes cause the latter to begin again. Cold, however, diminishes the irritability of the accelerators, as was observed by Baxt.

Effect of section of the accelerator nerves upon the duration of systole and of diastole. — The duration of systole and diastole was determined in the manner described by Hürthle[1] from the curve of carotid pressure recorded by the spring manometer. As this method will be referred to frequently in later parts of this paper a few words will be said about it here. Hürthle has shown that when tracings are taken with his manometers from the cavity of the left ventricle and from the aorta simultaneously the time elapsing between the appearance of the primary and the dicrotic waves of the aortic pulse curve is almost the duration of systole as determined from the curve of intraventricular pressure. Of course these curves do not coincide in time; the primary elevation in the curve of aortic pressure does not appear until after the beginning of the systole of the ventricle and the dicrotic wave does not appear until a short time after the end of the systole. The former period, that is, the time elapsing between the beginning of the systole and the occurrence of the primary elevation on the curve of aortic pressure, corresponds to the time during which the intraventricular pressure is rising, but is not high enough to force open the semilunar valves (the *Anspannungszeit*); the latter period corresponds to the interval which elapses between the closure of the semilunar valves and the appearance of the dicrotic wave. Hürthle found in a number of observations that these two periods were almost of equal length, and that therefore the duration of systole can be determined directly from the curve of aortic pressure by measuring the time interval between the primary and the dicrotic wave. He suggests that the duration of systole and diastole can be determined in a similar manner from the curves obtained by his manometer from other large arteries.

I have made use of the above method for determining the duration of systole and diastole in a number of experiments. A Hürthle spring manometer was connected with one of the carotids and the pulse recorded upon the smoked paper of a Hürthle kymograph; the speed of the latter was 100 mm. per second. A cannula was also connected with the femoral artery and the blood pressure recorded by a mercury manometer in the usual manner. In a number of experiments records of intraventricular and of aortic pressure were taken simultaneously, a spring manometer being connected with a catheter which had been passed down one carotid into the left ventricle,[1] while a second manometer was connected with the other carotid. Comparison of the curves obtained in this manner sometimes showed a somewhat greater discrepancy than was to be expected from Hürthle's description; still the differences were so slight that the error could as a rule be disregarded.

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[1] Hürthle: Archiv f. d. ges. Physiol., 1891, xlix, pp. 65-67.

One difficulty in using the above method for determining the duration of systole and diastole should be mentioned: it not unfrequently happens that after some change in the heart rate or blood pressure (such, for example, as that following stimulation of the cardiac nerves) the dicrotic wave of the curve becomes so indistinct as to make exact measurements difficult or impossible. Most of the experiments, however, or at least parts of most of the experiments, gave very satisfactory results.

The following experiment shows the effect upon the duration of systole and diastole of cutting the accelerator nerves.

Experiment C. — Small dog, anæsthetized by sulphate of morphine and ether; curare. Stellate ganglia exposed. Left femoral artery connected with mercury manometer; left carotid with Hürthle's spring manometer.

Time.				Heart beats in 10 seconds.	Duration in seconds of		Blood pressure mm.
Hr.	min.	sec.			systole	diastole	
1	23–	0		30	0.165	0.165	46
		10	Branches of l. stellate ganglion cut.				
	24–	0		29½	0.165–	0.170	46
	30–	0		28+	0.160–	0.190	42
	32–	0		28	0.160+	0.185	34
		10	Branches of r. stellate ganglion cut.				
		20		28–	0.160+	0.180	
		25			0.165	0.195	
		30		26+	0.175	0.195	
	33	0			0.205	0.225	
		30		21½	0.230	0.245	
	34			20½[2]	0.235+	0.270–	32
	35				0.250	0.270+	31

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[1] A silver male catheter was found most convenient for this purpose.

[2] The maximum heart rate caused by stimulation of the accelerators in this experiment was 31 beats in 10 seconds, *i. e.*, but very slightly greater than the heart rate before the nerves were divided. This was, therefore, one of the cases in which the accelerators were in a condition of almost maximal acceleration when they were cut.

The results obtained in the above experiment are better expressed in the form of a curve (Fig. 1) in which the ordinates represent the duration in 0.05 of a second of the systole and diastole of a single heart-beat at the end of each thirty seconds and the abscissæ represent periods of thirty seconds.

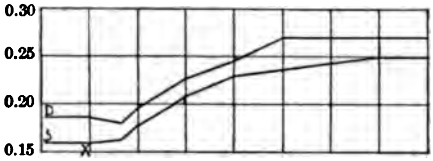

FIGURE 1. Experiment C. Accelerator nerves cut at *x*. *S* shows the duration of systole, *D* that of diastole in 5 hundredths of a second. The abscissæ represent periods of 30 seconds.

Parts of the tracing taken with the Hürthle manometer are reproduced in Fig. 2.

Inspection of the above table and curves shows that in this experiment section of the accelerators caused both systole and diastole to be prolonged, the prolongation of the former being relatively (*i. e.* as compared with the previous rate) slightly greater than that of the latter. In nearly all of my experiments results similar to these were obtained; usually however the prolongation of the systole, though a constant result of cutting the accelerators, was not so marked as in this case.

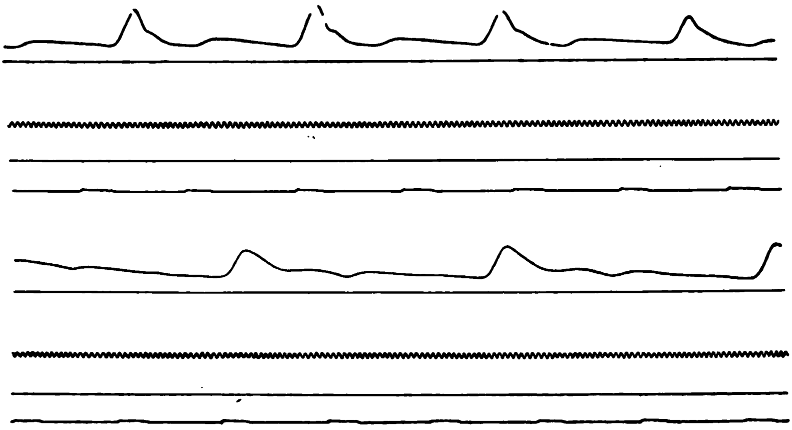

FIGURE 2. Two thirds the original size. Experiment C. Upper tracing before, lower tracing 2 minutes after, section of accelerators on right side. Time in intervals of 0.2 and 0.01 seconds.

The above experiment and the one quoted on p. 399, illustrate a point which I discussed in my earlier paper, namely, that section of the accelerator nerves on the right side usually causes a more marked slowing than section on the left side.[1]

Effect of section of the accelerator nerves upon the conduction of impulses from auricle to ventricle. — At least two experiments have been described which tend to show that the accelerator nerves contain fibres which affect especially the conduction of impulses from auricle to ventricle.[2] Certain observations have led me to think that these fibres are, under some circumstances at least, in tonic activity; or perhaps it would be better simply to say that the tonic activity of the accelerators affects the conduction of impulses from auricle to ventricle as well as the rate of the heart.

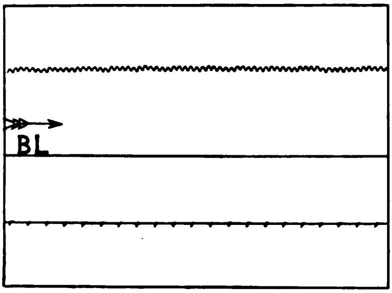

FIGURE 3. Two fifths original size. Experiment J. Curve of blood pressure before the accelerators were cut. *B L*, line of atmospheric pressure. Time in intervals of one second.

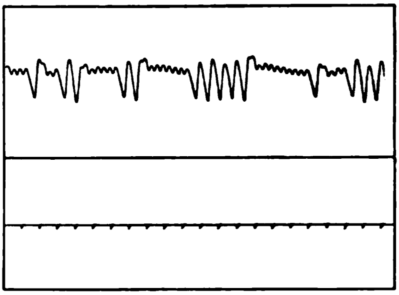

FIGURE 4. Two fifths original size. Part of the curve of Experiment J following Figure 3. Taken 4½ minutes after the branches of the right stellate ganglion were cut.

The following is one of a number of experiments from which this conclusion is drawn.

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[1] In most of my experiments in which the accelerators were stimulated on both the left and right sides, the latter caused greater acceleration than the former. Stricker and Wagner (*op. cit.*, p. 109) made the same observation, but François-Franck (Travaux du laboratoire de Marey, 1878–1879, iv, p. 83) failed to find any such differences in all but two of his experiments, — in these stimulation of the right accelerators caused a greater effect. These observations show that the distribution of the nerves varies in different individuals; but from my experiments I am convinced that, as a rule, more accelerator fibres pass to the heart on the right than on the left side.

[2] BAYLISS and STARLING: Journal of physiology, 1892, xiii, p. 407; HUNT and HARRINGTON: Journal of experimental medicine, 1897, ii, p. 725. In the present series of experiments I have often observed results similar to those described by Bayliss and Starling, namely, that stimulation of the accelerators after these nerves had been stimulated a number of times caused an acceleration of the auricles, but that the ventricles frequently failed to follow all of the auricular beats.

Experiment J. — Bitch, weighing 22.5 kilo. Anæsthesia produced by 4.8 grams acetone chloroform (trichloride of acetonic acid) and a little ether. Curare. Vagi cut. A catheter had been passed down one carotid into the left ventricle and a record of intraventricular pressure taken. The right stellate ganglion and its branches were exposed. The blood pressure was 57 mm. of mercury; the pulse rate 30 in 10 seconds.

The accompanying curves (Figs. 3 and 4) show the effect upon the heart rate of cutting the accelerator nerves on the right side. The heart had been beating very regularly before the accelerators were cut; soon after they were cut there began to appear, occasionally, beats of unusual length. The number of these long beats increased very rapidly until finally there were periods of several seconds' duration in which the heart was beating at but one half its previous rate. The record of intraventricular pressure showed that this irregularity of the heart resulted from the "dropping" of some of the ventricular beats; that is, the duration of one of these long beats was just twice that of a normal beat. In some cases there was a slight, sudden rise of short duration of the intraventricular pressure during these long beats; this probably was due to the auricular beat.[1]

The vagi had been divided in this experiment so that the change in the heart rate cannot be regarded as a reflex effect nor can it be attributed to changes in the blood pressure, for none occurred.

The auricular beats were not recorded, and therefore the following explanation of this irregularity cannot be considered as established with absolute certainty. But all observations seem to agree in showing that the ventricle when it suddenly begins to beat at one half its previous rate is responding to but every second auricular beat; the most probable explanation of the cardiac irregularity in the above experiment would seem to be that section of the accelerators had caused a diminution of the irritability of the muscle fibres connecting the auricle and ventricle. Of course another explanation may be offered, namely, that section of the accelerators had simply diminished the irritability of the ventricle so that it was unable to respond to all the impulses reaching it from the auricles. I think however that in the light of the work of Gaskell, MacWilliam, and Bayliss and Starling upon the conduction of impulses from the auricle to the ventricle, the former explanation is the more probable.

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[1] Cf. HÜRTHLE: Archiv f. d. ges. Physiol., 1891, xlix, p. 55.

In other experiments upon dogs and cats and in one upon an opossum similar irregularities of the heart occurred after section of the accelerators, but as a rule they were less marked than in the experiment just described; in some, for example, a ventricular beat was dropped only occasionally. It is interesting that in all these cases it was section of the accelerators on the right side which led to these irregularities.

Inasmuch as the mammalian heart will continue under proper conditions to beat with great regularity for hours after it is entirely separated from the central nervous system, the question may be raised why in the above experiments section of the accelerators caused it to beat irregularly. It is quite probable that in these cases the irritability of the heart had been decreased in some manner to such an extent that the stimulus afforded by the accelerators was necessary to maintain regular cardiac action. In experiment J, for example, there were a number of influences which may have affected the heart. Thus the anæsthetic employed, acetone chloroform, seems to cause a loss of irritability of the heart, for in animals to which this drug has been given the heart rate is usually slow and the beat weak[1] after section of all the cardiac nerves; moreover, the blood pressure was low and perhaps the heart had been injured by the sound passed into the left ventricle.

In the other experiments also in which section of the accelerators led to cardiac irregularity there were conditions which make it probable that the irritability of the heart was abnormally low.

If the explanation offered above is the correct one, we should expect stimulation of the accelerators to cause the cardiac irregularity to disappear, especially as Bayliss and Starling[2] have shown that stimulation of these nerves makes the transmission of impulses from auricle to ventricle more easy; as a matter of fact electrical stimulation of the accelerators not only caused an increase of the heart rate but an entire disappearance of the irregularity. The latter also followed the intravenous injection of hot normal saline solution, an agent which undoubtedly increases irritability of the heart.

It is also interesting to note that in some of the exceptional cases in which section of the accelerators showed that they were not in tonic activity the heart was irregular both before and after all the cardiac nerves were divided.

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[1] The injurious action of acetone chloroform upon the heart may be due to impurities which are usually found in specimens of this drug.

[2] BAYLISS and STARLING: Journal of physiology, 1892, xiii, p. 414.

From such observations and considerations as the above I think the conclusion may safely be drawn that at times the tonic activity of the accelerators is very important for the regular action of the heart.

FATIGUE OF THE ACCELERATOR NERVES.

The statement is frequently made that the accelerator nerves are not easily fatigued, but the only basis for this assertion with which I am acquainted is an observation by Böhm.[1] This investigator stated that he had stimulated the accelerators for two minutes without observing any indication of fatigue and expressed a doubt as to whether they can be fatigued at all. When it is remembered that even in cases of maximal acceleration the heart rate is rarely doubled, it does not seem reasonable to suppose that if any fatigue did occur it would be observed when these nerves were stimulated for so short a period as two minutes.

That these nerves are not easily fatigued when subjected to a moderate stimulation follows from the fact that they are in a condition of tonic activity; on the other hand it will be shown that fatigue may readily occur when the nerves are subjected to strong electrical stimulation. My experiments bearing on this point will be discussed in two parts; the first will be entirely descriptive, in the second an effort will be made to ascertain so far as possible where the fatigue occurs.

Changes in the rate of the heart-beat. — When the accelerator nerves are stimulated continuously for some time (ten minutes for example) the heart does not remain long at the maximum rate but shows a tendency to return to the rate at which it was beating before the stimulation began. If these changes in the heart rate be expressed in the form of a curve in which the abscissæ represent the time and the ordinates the number of heart-beats in a given time (*e. g.* ten seconds) it will be found that as a rule the decrease in the heart rate occurs in two periods. There is first a comparatively rapid fall in the curve; this is followed by a much longer period in which the descent is very slight. Under some circumstances — these being determined by the condition of the heart and nerves, the character of the stimulus employed, etc. — the curve is continued in an almost straight line during the second period; in other words, the heart continues beating throughout this period at nearly a constant rate. The duration of these two periods is very variable; occasionally they are absent altogether, there being in this case either no decrease at all in the heart rate (if the stimulation is of comparatively short duration) or the heart rate may decrease regularly; *i. e.*, the curve becomes an almost straight line.

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[1] BÖHM: Archiv f. exper. Pathol. u. Pharmakol., 1875, iv, p. 275.

The following experiment and curve show the usual result of stimulating the accelerators continuously for some time.

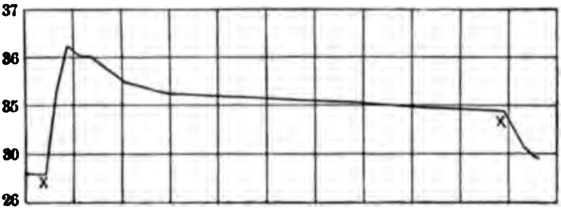

FIGURE 5. Experiment 117. Stimulation of the accelerator nerves for 9½ minutes (*x* to *x*). The ordinates represent the number of heart-beats in 10 seconds; the abscissæ periods of one minute.

Experiment 117. Small dog. Morphine and ether. Accelerators and vagi cut. The accelerators had been stimulated a number of times with results very similar to those shown in Fig. 5.

Curves similar to the above are nearly always obtained when the accelerators are stimulated, whatever the condition of the heart or the strength or character of the stimulus employed. The same form of curve is also obtained in whatever part of their course the nerve fibres are stimulated, whether in the spinal cord, the rami communicantes, the annulus of Vieussens, or the small nerves running directly from the inferior cervical or the stellate ganglion to the cardiac plexus. When the same nerve is stimulated a number of times in succession there is a gradual lowering of the height of the curve, but the form remains the same; the latent period also becomes longer with the successive stimulations.

The above results recall those obtained when the peripheral end of the vagus is stimulated: in this case the heart, after a short period during which it is stopped or slowed, tends to return partially to its previous rate; it often reaches a constant rate, at which it continues to beat for hours.[1] Martin[2] found that when the isolated mammalian heart is warmed the beat is at first increased in frequency, but presently the heart returns partially to the former frequency and continues beating at this slower rate; in fact, if his results were expressed as a curve, the latter would have very nearly the same form as that obtained when the accelerator nerves are stimulated. Martin also called attention to the fact that similar changes in the heart rate are observed in some cases of fever — that is, the rate tends to return to the normal although the temperature remains the same.

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[1] See HOUGH: Journal of physiology, 1895, xviii, p. 176; also LAULANIÉ: Comptes rendus de l'académie des sciences, 1889, cix, p. 408.

[2] MARTIN: Physiological papers, 1895, p. 104.

When the accelerators are stimulated in the course of a long continued stimulation of the vagus, the curve of fatigue is the same as when they are stimulated alone.[1]

Effect of the strength of the stimulus upon the course of fatigue. — The effect upon the heart in any given case of stimulating the accelerators with currents of varying strength and rate seems to be determined largely by the condition of the heart and nerves at the time of stimulation. If the heart is vigorous and the nerves have not been stimulated often it may be said that in general the stronger the current, the longer continued is the phase of maximal acceleration; if on the other hand the heart is not very vigorous or the nerves have been stimulated a number of times, fatigue occurs more quickly with a strong than with a weak current although the maximal rate reached by the heart may be the same in both cases. Also as a rule the stronger the stimulus the higher is the level at which the heart continues to beat during the stimulation.

It is interesting that a slight initial decrease in the heart rate occurs when the accelerators are stimulated with a current too weak to cause a maximum acceleration; this resembles the "escape" observed by Hough when slight slowing was caused by stimulation of the peripheral end of the vagus.

Effect of the rate of stimulation upon the fatigue of the accelerators. — The rate of the stimuli applied to the accelerators seems to have a greater effect upon the course of fatigue than does their strength. This was shown in many experiments in which the primary circuit of the du Bois-Reymond induction coil was interrupted by Ludwig's *Schlagwähler* or by an "oscillating rod" kept in vibration by an electromagnet; the number of stimuli per second could be varied within wide limits by either of these instruments.

The following experiment shows how much more quickly fatigue is produced with a rapid rate of stimulation than with a slower rate.

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[1] An experiment illustrating this is given on pages 174 and 175 of my article in the Journal of experimental medicine, 1897, ii.

Experiment 187. — Dog. Morphine and ether. Curare. Spinal cord cut in mid cervical region. Branches of stellate ganglia divided. The result of stimulating the accelerators is shown in the following table.

Time.			Heart-beats
Hrs.	min.		in 10 seconds.
3	41		11
		R. annulus stimulated; primary circuit interrupted 5+ times per second; secondary coil 13 cm.	14–
			21½+
			20½
			19–
			16½
	42		—[1]
		Stimulus off annulus.	16½
			—
			16½–
			16½
			16
3	53		
		R. annulus stim.; 2½ stimuli per second; secondary coil 13 cm.	12–
			—
			16+
			—
			17–
			—
	55	Secondary coil 10 cm.	17+
			—
			20+
	56		20+
		Coil 5 cm.	—
			—
			20+
	59		20–
		Coil 0 cm.	—
			—
			20
4	2		20
		5 stimuli per second; coil 0 cm.	—
			21–
			—
			18+
	4		17½
			17

In the above experiment no diminution of the acceleration occurred when the accelerators were stimulated with 2½ stimuli per second, whereas 5+ stimuli per second caused such a diminution to occur very quickly.

In almost every experiment there was found a rate at which the accelerators could be stimulated for some time with but little decrease in the acceleration. This, which may be called the optimum rate, varied greatly in different experiments and seemed to be determined by the condition of the heart and nerves. Thus in one experiment stimulation of the right annulus with an ordinary du Bois-Reymond coil in which the primary circuit was interrupted by the Neef hammer, caused the heart rate to increase from 23 to 29 beats in 10 seconds; but in the course of three minutes during which the stimulation continued the rate decreased to 24½+: after a few minutes' rest the annulus was again stimulated but now the primary circuit of the coil was interrupted by the *Schlagwähler* at the rate of 6 times per second; the rate of the heart increased from 22 to 29½ beats in 10 seconds; after three minutes' stimulation the rate was 29, showing that no fatigue had occurred.

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[1] The dash (—) indicates that the heart-beats were not counted for 10 seconds.

Duration of systole and diastole in fatigue. — A number of experiments was made to determine whether both systole and diastole became longer when fatigue occurred in the course of a prolonged stimulation of the accelerators; it was found that they were longer, and that the prolongation occurred to about the same extent in both cases, so that the curve representing these changes were almost parallel.

It is interesting to compare these results with those observed when the heart is "escaping" during a prolonged stimulation of the vagus. When the peripheral end of the vagus is stimulated the diastole is always prolonged; as a rule the systole is also prolonged, but to a less extent; but while the diastole becomes shorter as the heart "escapes" to a more rapid rate, the systole remains about the same length throughout the stimulation. Thus during the "escape" of the heart from slowing as well as during the slowing caused by stimulation of the vagus it is the diastole which is most easily affected. On the other hand when the heart is slowed by section of the accelerators or when it is accelerated by the stimulation of these nerves or when fatigue occurs during their stimulation, both systole and diastole are affected; in fact, it seems almost as if one was affected through the other. If however the accelerators are stimulated when the heart is beating very slowly, as a result for example of stimulation of the vagus, then shortening of the diastole may occur before that of the systole; this point will be referred to later.

Local effects in the nerve. — It became evident very early in the investigation of the cause of the decrease in the heart rate during a prolonged stimulation of the accelerators that two factors are involved; first, a local action upon the nerve at the point stimulated, and, secondly, an effect upon the heart itself or upon the nerve endings in the heart. The latter effect is the more interesting in this connection, but it is necessary to consider the former or a serious error will be introduced into the experiment.

Local effect upon the nerve at the point of stimulation.— Howell[1] has shown that when certain nerves, especially some of the non-medullated variety, are stimulated with the faradic current for some time there occurs a loss of irritability at the point of stimulation; he called this loss of irritability "stimulation fatigue," without however expressing any opinion as to its real nature.

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[1] HOWELL, BUDGETT, and LEONARD: Journal of physiology, 1894, xvi, p. 311.

This local effect of the current upon the nerve is easily demonstrated in the case of the accelerators. If the stimulating electrodes be held on one point of the nerve until the heart rate has begun to decrease and they then be moved a few millimetres nearer the heart, a second increase in the heart rate occurs; this, like the first acceleration, continues a short time and then the heart rate again decreases. This experiment may be repeated a number of times.

The following experiment will serve to illustrate this point.

Experiment 119. Dog. Morphine and ether; curare. Accelerators and vagi cut. Blood pressure 82 mm.

Time. Hrs.	min.	sec.		Heart-beats in 10 seconds.
5	20	30		28½
			Stimulation of r. annulus begun; secondary coil 17 cm.	
		40		32+
	21			40+
	22			37½+
	25			32½
	26			31
			Electrodes moved slightly.	
	26	20		36
	27			34½
			Electrodes moved slightly.	
		20		39+
	28			37½
	30			34
			Electrodes moved slightly.	
		20		38½

In the above experiment the electrodes were moved towards the heart, but the same result is obtained when they are moved farther away from the heart, — a fact which shows that the conductivity of the nerve fibres at the point of stimulation is not lost.

If instead of moving the electrodes to a different part of the nerve after the acceleration has begun to decrease, the strength of the stimulating current suddenly be increased, the pulse rate may again be accelerated for a short time. By increasing the strength of the stimulus repeatedly at short intervals the heart may be kept beating at the maximal rate for some time.

Effect of stimulating the accelerators in different parts of their course. — Howell has shown that it is in the non-medullated nerve fibres that this local loss of irritability most often occurs, while the medullated fibres are less subject to it. In view of these results it is of interest to compare the effects of stimulating the accelerator nerves in that part of their course in which they are medullated with those observed when they are stimulated at a point where they are non-medullated. According to the prevailing view, these nerve fibres belong to the medullated variety while passing from the spinal cord through the rami communicantes to the stellate, inferior cervical, and perhaps other ganglia of the sympathetic system; from these ganglia they are continued as non-medullated fibres to the cardiac plexus. According to this view, therefore, in order to compare the effects of stimulating the medullated and non-medullated accelerator fibres we have only to stimulate the spinal cord or the rami communicantes on the one hand and the small nerves passing from the annulus and the inferior cervical ganglion on the other. The annulus itself probably contains both medullated and non-medullated fibres.

It has been already stated that the form of the curves of fatigue was the same in whatever part of their course the nerves were stimulated; it is impossible in most of the experiments, however, to state whether the fatigue occurred in the heart or was a local effect upon the nerve, as at the time no special attention was given to this point. The following experiment indicates, however, that the local effect upon the nerves varies according to the part of the course in which they are stimulated.

Experiment 125. Dog. Morphine and ether. Right accelerators cut. Vagi intact.

The second thoracic ramus communicans had been stimulated a number of times, during and probably as a result of which the heart rate had decreased from 34 to 22 beats in 10 seconds. The right annulus was now stimulated for the first time, with the result shown in the following table.

Time. Hrs.	min.	sec.		Heart-beats in 10 seconds.	Time. Hrs.	min.	sec.		Heart-beats in 10 seconds.
12	40	30		22½−		43			26½
			R. annulus coil 16 cm.			46			25
				26½+			40	Current turned to 2d ram. com.	
				35−		47			36
				35+		48			34½+
				35+		49			33+
	41	30		31−		52	40		30½
	42			27+					
		30		26½+					

Thus during a stimulation of the right annulus continuing 6 minutes the heart rate fell to 25 beats in 10 seconds, while during a stimulation of the ramus communicans of the same duration it fell to but 30½ and this notwithstanding the fact that the latter nerve had been stimulated a number of times while the former was stimulated for the first time and that also a much larger number of accelerator fibres was doubtless stimulated when the current was applied to the annulus than when the ramus was stimulated. The explanation of the above difference is probably to be found in the fact that the nerve fibres in the ramus are medullated while many at least of those in the annulus are non-medullated.[1]

There is also a difference in the rapidity with which the different accelerator nerve fibres recover their irritability after stimulation. Thus in the above experiment stimulation of the annulus after 20 minutes' rest caused an acceleration of from 23 to but 26 beats in 10 seconds, whereas stimulation of the second ramus after a rest of 20 minutes caused the heart rate to increase to 35 in 10 seconds.

Fatigue occurring in the heart as a result of stimulating the accelerators.—While the experiment described above shows that the decrease in the heart rate occurring after a prolonged stimulation of the accelerators is due in part to a local action upon the nerve fibres at the point of stimulation, there is very clear evidence that the heart itself can be fatigued by excessive stimulation of these nerves. That the heart is not easily fatigued by stimulation of the accelerators follows from the fact that they are usually in a condition of tonic activity; it was also shown above that moderate electrical stimulation, especially stimulation with a weak, slowly interrupted current, does not easily cause fatigue. If, however, the electrical stimulation is excessive or often repeated, and especially if the heart has been subjected to injurious influences, distinct indications of fatigue in the heart[2] occur, as will be shown below. It is very probable that the same thing occurs whenever the accelerators are thrown into excessive action by causes originating in the body itself.

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[1] Similar results were obtained in a few experiments in which the spinal cord and the accelerator nerves were stimulated; most of these experiments, however, were complicated by an unexpected factor. In a number in which the spinal cord had been divided in the cervical region not only was the acceleration from stimulation of the cord and of the accelerators very slight, but fatigue occurred with extraordinary rapidity. The section of the cord seemed to reduce the irritability not only of the accelerator fibres in the cord, but also of those in the annulus and the branches of the stellate ganglia. Munk and Schultz (Archiv für Physiologie, 1898, p. 307) have observed a similar loss of irritability in the phrenic nerve after section of the spinal cord; one explanation which they suggest, with considerable reserve, is that the irritability of the nerve was affected by the changes in the circulation following section of the cord. It is doubtful whether this explanation would hold for the accelerators; for, as has been pointed out above, these nerves are not easily affected by changes in the blood pressure.

[2] The expression "fatigue in the heart" is meant to include either fatigue of the endings of the accelerator nerves or of the cardiac muscle, or of both; it is by no means easy in many cases to distinguish between the two.

Effect of repeated stimulations of the accelerators upon the pulse rate, irritability of the heart, etc.—After repeated stimulation of the accelerators the pulse rate very often decreases. This decrease frequently occurs in cases where the vagi have been cut and therefore it cannot always be referred to an increased influence of the inhibitory nerves; nor can it in many cases be due to changes in the blood pressure or to a decrease in the animal's temperature, for it takes place when no such changes occur. Moreover in many experiments no change in the heart rate occurs during periods of rest of the same duration as the periods of stimulation. From such considerations as these I think the conclusion must be drawn that the slowing of the heart following stimulation of the accelerators is due to fatigue of the heart itself.

The following data may serve as an illustration of the extent to which the heart may be slowed as a result of repeated and long continued stimulation of the accelerators. The experiment was performed upon a dog anæsthetized by morphine and ether. After section of the vagi and accelerators the heart rate was 40+ in 10 seconds. The accelerators were stimulated 39 minutes; soon after the stimulation the rate was 34 in 10 seconds and it remained at this level during the period of rest which followed the stimulation. After a second stimulation of the accelerators continuing 9 minutes the heart was beating at the rate of 28 in 10 seconds and continued at this rate during a period of 5 minutes' rest. The nerves were now stimulated a third time. The stimulation continued only five minutes, but after it the rate sank to 22½. During this entire period the blood pressure had fallen very slightly, only about 10 mm. of mercury.

Results similar to the above are often observed, but it is not always so easy to exclude factors other than that of the stimulation of the accelerators.

That the irritability of the heart is lowered by long continued stimulations of the accelerators is shown by such an experiment as the following; the animal used was a dog anæsthetized by morphine and ether. Early in the experiment a small branch of the right annulus was stimulated for 20 seconds; the heart rate increased from 20 to 33 in 10 seconds. The ventral limb of the right annulus was now stimulated for 21 minutes, the position of the electrodes upon the nerve being frequently changed. After the cessation of the stimulation the small branch of the annulus mentioned above was stimulated again with the same strength of current as before: the heart rate increased from 20 to but 25½ in 10 seconds. The only plausible explanation of the slight effect caused by the second stimulation of this small nerve is that the irritability of the heart had been diminished by the long continued stimulation of the annulus.

Numerous other cases could be quoted in which stimulation of the annulus or of the small branches passing from the inferior cervical ganglion had but little effect upon the heart after repeated or long continued stimulation of the spinal cord or of those rami communicantes through which the accelerator fibres pass.

Another indication that stimulation of the accelerators causes a diminution of the irritability of the heart is found in the study of the effects of the intravenous injection of extracts of the suprarenal gland. As is well known, the injection of such extracts into an animal in which all the cardiac nerves have been cut leads to a great acceleration of the heart-beat; in a number of my experiments the maximum rate reached after such an injection coincided almost exactly with the maximum rate resulting from stimulation of the accelerators. Small quantities of the extract may be injected a number of times in succession without any diminution of the effect. If, however, the accelerators be stimulated for some time with a strong current between two such injections, it is found that the second injection has less effect than the first one. Thus, early in one experiment stimulation of the right annulus caused an acceleration from 31½ to 40½ beats in 10 seconds, while the injection of an aqueous extract of the suprarenal of a dog caused the heart rate to increase to 39. The accelerators were now stimulated for about an hour, and then after a period of rest they were again stimulated for a short time; the result of the second stimulation was that the heart rate increased from 31 to 35½ beats in 10 seconds; the injection of the same quantity of the suprarenal extract as above caused the heart rate to increase to 35.[1]

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[1] The interpretation of these results will depend upon the view held as to the action of suprarenal extracts upon the heart. If we accept the view of v. Cyon (Archiv f. d. ges. Physiol., 1898, lxxii, p. 371) that these extracts act upon the endings of the accelerator nerves, these experiments can only be regarded as evidence that the stimulation of the accelerators caused fatigue of the endings of these nerves; if, however, we adopt the view of Cleghorn (This journal, 1899, ii. p. 281), based upon experiments with the isolated apex of the dog's heart, that the

In another experiment suprarenal extract caused a marked acceleration before the accelerators were stimulated; after the nerves were stimulated until they ceased to have any effect upon the heart another injection of the suprarenal extract was made, but with entirely negative results.[1] At times on the other hand the injection of suprarenal extract seems to restore the influence of the accelerators over the heart.

Two other results of repeated stimulations of the accelerators which probably indicate a loss of irritability of the heart may be mentioned: first, the latent period of acceleration is prolonged, and secondly, the after-effect of the stimulation is much less marked, *i. e.*, the heart returns much more quickly to its previous rate after stimulation of the accelerators.[2] This rapid return of the heart to the

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action of these extracts is upon the cardiac muscle itself, then I think my experiments can be regarded as evidence that the heart muscle is fatigued by stimulation of the accelerators.

[1] There are other points of similarity between the action of the accelerators and suprarenal extracts. Thus it has been my experience that when stimulation of the former from any cause failed to cause an acceleration of the heart, injection of the latter was also without effect. The effect of repeating the injections at short intervals is, moreover, very much like that of moving the electrodes slightly during a long continued stimulation of the nerves; after each injection, as after each shifting of the electrodes, there is a fresh increase in the heart rate. The heart can be kept beating at the maximum rate for some time in this manner.

It is also interesting to note in this connection that it was impossible to obtain any summation of the effect of the maximal stimulation of the accelerators and of the injection of suprarenal extracts; thus, when the injection was made simultaneously with the stimulation, the maximum rate of the heart was no greater than when it was made alone or when the accelerators were stimulated alone. If, however, the stimulus applied to the accelerators was sub-maximal, the injection of suprarenal extract would cause the heart rate to reach the same level as when the stimulus was maximal or when a considerable amount of extract was injected alone. There seemed to be a limit to the rate to which the heart could be accelerated (just as in cases of Basedow's disease, fever is said to cause no farther acceleration of the heart); in these experiments this limit was the same for the injection of suprarenal extract and for the electrical stimulation of the accelerators. It may be added that in one experiment injection of warm Ringer solution caused the same acceleration as did stimulation of the spinal cord and of the annulus.

Böhm states that in cats certain drugs cause a greater increase in the heart rate than does stimulation of the accelerators, and that therefore the acceleration of the latter case is not maximal; it should be noted, however, that these comparisons were not made upon the same animal.

[2] Böhm (Archiv f. exper. Pathol. u. Pharmakol., 1875, iv, p. 275) made a similar observation, but did not offer any explanation of it.

previous rate is especially marked if the irritability of the heart has been reduced by the action of cold as well as by repeated stimulation of the accelerators.

Stimulation of the vagus after repeated stimulation of the accelerators. — I think further evidence of fatigue occurring in the heart as a result of stimulating the accelerators is to be found in the comparison of the effects of stimulating the vagus before and after repeated stimulations of the accelerators. We know from the experiments of Hough[1] (and I have had many opportunities of confirming his results) that the less vigorous the heart the greater is the effect of the stimulation of the vagus. Hence if stimulation of the accelerators does reduce the vigor of the heart we should expect to find the effect of the vagus increased after a long continued stimulation of these nerves; experiment shows this to be the case. This result is shown in a striking manner if, as was the case in the following experiment, the accelerators are stimulated during a prolonged stimulation of the vagus.

Experiment of July 2d. Bitch. Morphine and ether; curare. Vagi and accelerators cut. The accelerators had been stimulated a number of times with the result that the maximum acceleration was less with each successive stimulation. The following table shows that the effect of the vagus became greater after stimulation of the accelerators.

Time.				Heart-beats
Hrs.	min.	sec.		in 10 seconds.
4	22			40
			Stimulation of r. vagus begun; 13 stimuli per second; secondary coil 0 cm.	
		10		27
		20		23+
	23			25
			Stimulation of r. annulus begun; secondary coil 17 cm.	
		10		32½
		20		41
		30		41½
	24			36½
	25			31
			Stimulus off annulus.	
	26			24+
4	26	10		24
		30		21
			R. annulus stimulated; secondary coil.	
	27	10		24½+
	28			23½
			Stimulus off annulus.	
	29			18
			Stimulus off vagus.	
	35			38

In this experiment the slowing of the heart caused by stimulation of the vagus became greater after each stimulation of the accelerators. That this was due to a change in the heart resulting from the stimulation of the accelerators is shown by the fact that when the vagus was stimulated alone the heart rate tended to return to the normal rather than to become slower; an indication of this tendency is shown in the first part of the above table.

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[1] HOUGH: Journal of physiology, 1895, xviii, p. 162.

In another experiment very similar to the above, stimulation of the vagus caused the heart to be slowed from 27 to $17\frac{1}{2}$ beats in 10 seconds; after a stimulation of the accelerators of but 28 seconds' duration stimulation of the vagus caused the heart to be slowed to 11 beats in 10 seconds. It should be added that in order to show the greater efficiency of the stimulation of the vagus after stimulation of the accelerators the former must follow the latter immediately, that is, the diminution of the irritability of the heart is of short duration unless the stimulation of the accelerators is continued for a long time.

If the vagi are intact and in tonic activity the slowing following stimulation of the accelerators seems to be due in part to an increased influence of the vagi over the heart, for the slowing occurs much more quickly in experiments in which the vagi are intact than in those in which they have been divided.

Such experiments as those just described seem to me to be of special interest and importance since they seem to show very clearly that stimulation of the accelerators causes diminished irritability of the cardiac muscle and not simply fatigue of the endings of the accelerator nerves; it is very difficult in most cases in which there are indications of fatigue in the heart to distinguish between these two possibilities.

Effect of some drugs upon fatigue of the accelerators. — The results of a few observations which I have made incidentally upon the effect of two or three drugs upon the occurrence of fatigue from stimulation of the accelerators may be referred to here.

After the intravenous injection of large doses of atropine and curare stimulation of the accelerators causes very rapid fatigue; whereas after the injection of sodium iodide [1] the fatigue is much less marked and the same is apparently the case after the injection of extracts of the suprarenal glands.

The following experiment illustrates the effect of a large dose of curare and of the injection of sodium iodide.

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[1] The use of sodium iodide was suggested to me by the work of Barbèra (Archiv f. d. ges. Physiol., 1897, lxviii, p. 436) and v. Cyon (*Ibid.*, 1898, lxii, p. 176), who found that this salt acts as a powerful stimulus to the vasomotor and accelerator nerves; it also increases the irritability of these nerves while markedly lowering that of the vagi.

Experiment 148. Dog. Morphine and ether. A very large amount of curare was injected into the femoral vein as a result of which the blood pressure had fallen from 83 to 20 mm. of mercury: it rose later to 22 mm. The accelerators on the right side had been divided. The results are given in the following table.

Time.			Heart-beat in 10 seconds.	Blood pressure.
Hrs.	min.			
1	50		21+	22
		R. annulus stim. for 70 sec., coil 14 cm.		
			33	
			32	
			30	
			25½	
			23	
		(Record not good for 20 seconds.)	?	?
		Stimulus off annulus.		
			19	
	53		20	21
		5.2 ccm. 20% sol. NaI injected into femoral vein.		
	54		20	19[1]
		R. annulus stim. as above.		
			27	
			32	
			?	
			29½	
			29+	19
			29+	
1	55		29+	
			29+	
		Stim. off annulus.		
			27½	
			26½	
	56		28	

The experiment was continued for 25 minutes, during which the accelerators were stimulated a number of times with results very similar to the above, *i. e.*, there were very slight indications of fatigue.

The fatigue caused by stimulation of the accelerators is also less after the intravenous injection of warm Ringer solution; the acceleration is also greater. Thus in one experiment stimulation of the spinal cord, which had been divided in the upper cervical region, caused an acceleration from 19 to 25 beats in 10 seconds but this rate was maintained for but a very short time. After the intravenous injection of 400 c.c. of warm Ringer solution, which caused the blood pressure to increase from 27 to 61 mm. of mercury, stimulation of the cord caused an acceleration to 29 beats in 10 seconds and there was very little evidence of fatigue when the stimulation was continued for some time. Of course the greater efficiency of the stimulation of the accelerators may have been due in part to the higher blood pressure.

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[1] As a rule the injection of sodium iodide causes first a fall and then a rise of blood pressure; that this did not occur in this experiment was probably due to the action of the very large dose of curare.

A large amount of atropine suddenly injected into a vein causes the heart (or the endings of the accelerators) to be fatigued by stimulation of the accelerators almost as rapidly as did curare in the above experiment.

Death from stimulation of the accelerators. — It happened not unfrequently that death resulted from stimulation of the accelerators in these experiments. This usually occurred after a long experiment in which the accelerators had been stimulated repeatedly with strong currents or after the irritability of the heart had been reduced by the action of curare or of cold. Sometimes the ventricle suddenly went into fibrillar contractions either during or immediately after the stimulation of the accelerators, in other cases the heart-beats became slower and weaker until they were no longer recorded.

An example of the latter mode of death is the following.

Experiment 120. Small dog. Morphine and ether. A very large amount of curare was injected into the femoral vein, as a consequence of which the blood pressure fell to 27 mm.

The results of stimulating the accelerators are shown in the following table.

Time. Hrs.	min.	sec.	Heart-beats in 10 seconds.	Time. Hrs.	min.	sec.	Heart-beats in 10 seconds.
3	45		24½		48		25
			R. annulus stimulated; coil 14 cm.		50		23–
					52		20
		20	37		54		12½–
		30	38½		55		12½
	47		33		56		11

The heart became so weak that the beat was not recorded; it soon stopped altogether.

Death caused by fibrillar contractions, together with the effect of cold on the heart, is well illustrated by the following experiment.

Experiment 147. Small bitch. Morphine and ether. Vagi cut. A small opening was made in the pericardium and a considerable quantity of normal saline solution at about 10° C. injected; the heart was slowed slightly for a short time and then it returned to its previous rate (31 in 10 seconds).

Time. Hrs.	min.	sec.		Heart-beats in 10 seconds.
3	54			31
			Branches of r. stellate ganglion cut.	
				15½
	55			12
				12
	56			24
			Normal saline (10° C.) injected into pericardial cavity.	
				23½
				22½+
	57		Branches of l. stellate ganglion cut.	
		10		23
4	1			23+
			R. annulus stimulated; secondary coil 12 cm.	
				24+
				27½
				30+
				29+
				28
				24
			Ventricles going into fibrillar contractions; auricles beating regularly.	

Death from stimulation of the accelerators frequently occurred with as much suddenness as in the above experiment, although in those cases in which the accelerators had been stimulated repeatedly the heart rate had as a rule decreased somewhat (from 28 to 19 in one experiment, for example).

Sometimes the heart beat regularly and rapidly during a stimulation of the accelerators but died a few seconds after the stimulations ceased.

The strength of the current used in these experiments in which death occurred was no greater than that used in other experiments, and the nerves were usually stimulated near the stellate ganglion; it is very improbable therefore that death was due to an escape of current to the heart: it probably was due simply to the inability of the heart in its weakened and exhausted condition to respond to the demands made upon it by the stimulation of the accelerators.

RELATION OF THE VAGI TO THE ACCELERATORS; PROTECTIVE ACTION OF THE VAGI UPON THE HEART.

Stimulation of the vagus after section of the accelerators. — In my previous paper I have called attention to two effects of the section of the accelerators upon the results of stimulating the vagi; (1) the same stimulus applied to the vagus causes a greater slowing of the heart after the accelerators are cut, than before, and (2) the "escape" of the heart during a long continued stimulation of the vagus is less complete after the accelerators are cut.

Other effects of the section of the accelerators in relation to the stimulation of the vagus will be described later in this paper; one other point may be mentioned here although I have but few experiments to quote. A number of physiologists[1] state that while stimulation of the accelerators causes a shortening of both systole and diastole, stimulation of the vagus causes a prolongation of diastole and as a rule but little effect upon the duration of systole. In comparing the effects of stimulating the vagus in different experiments of my own I often have observed that in those cases in which the accelerators were intact and in tonic activity stimulation of the vagus had little effect upon the duration of the systole, while in those in which the accelerators had been cut a very considerable prolongation of systole frequently occurred. Thus in experiment C (see p. 402) after the accelerators were cut stimulation of the vagus caused the systole to be prolonged from 0.26 to 0.345 seconds or over 32 per cent.

Stimulation of the accelerators after section of the vagi. — The tonic activity of the vagi limits the effect of stimulating the accelerators; this is shown in two ways. In the first place stimulation of the accelerators with sub-maximal stimuli has a greater effect upon the heart after than before section of the vagi if these are in tonic activity. Thus in one experiment stimulation of the accelerators with a weak current caused the heart rate to increase from 16 to 24 beats in 10 seconds when the vagi were intact; section of the vagi caused the heart rate to increase to 26½; stimulation of the accelerators with the same strength of current as before caused the heart rate to increase to 34 in 10 seconds. With very strong stimuli, however, the acceleration is the same before and after section of the vagi, for now the effect of the vagi is completely overcome and the heart is accelerated to its maximum rate.

In the second place, the after-effect of stimulation of the accelerators is much more marked after section of the vagi; the heart returns more slowly to the previous rate. The following experiment illustrates this point.

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[1] Cf. HÜRTHLE: Archiv f. d. ges. Physiol., 1891, xlix, pp. 86–88.

Experiment M. Large dog. Morphine and ether. Curare. Accelerators cut.

Time. Hrs.	min.	sec.		Heart-beats in 10 seconds.	Time. Hrs.	min.	sec.		Heart-beats in 10 seconds.
1	51			20−				R. annulus stimulated for 10 seconds; coil 10 cm.	24
			R. annulus stimulated for 10 seconds; coil 10 cm.	22½					28
				23½+	1	58			26½
		30		19½+					23
	55		R. vagus cut.						21+
	57	30		21+			30		20½
									20

Antagonism of the accelerator and inhibitory nerves in their effects upon the duration of systole and diastole. — I have shown elsewhere that, contrary to the view of Baxt, when the accelerators and vagi are stimulated simultaneously, the effect upon the heart rate is determined by the relative strength of the two stimulating currents, and that for sub-maximal stimuli the result is approximately the arithmetical mean of the effects of stimulating the nerves separately.[1] Does this law of the antagonism of these nerves hold good for both systole and diastole? My experiments, which however have not been very numerous upon this point, indicate that this is the case. In such experiments as these it is necessary to use currents the strength of which can properly be compared with each other as regards their effect upon the heart; for while a relatively weak stimulation of the accelerators will cause a shortening of the systole, a stronger stimulation of the vagus is required to prolong this phase of the heart's contraction, and on the other hand a stimulus which, when applied to the vagus, will cause a very great prolongation of diastole may, when applied to the accelerators, affect neither systole nor diastole. In order to cause a prolongation of the systole by stimulating the vagus, it is sometimes necessary to use a current strong enough to bring the heart to a standstill; then, as the heart escapes from this standstill, the systoles are found to be prolonged.

If we accept a suggestion that has been made,[2] namely, that those nerve fibres which affect systole are different physiologically from those which affect diastole, the differences noted above may be explained by supposing that one set of fibres is much more irritable to electrical stimulation than the other.

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[1] HUNT: Journal of experimental medicine, 1897, ii, p. 171. These results have since been confirmed by v. Cyon (Archiv f. d. ges. Physiol, 1898, lxx, p. 244), Wertheimer (Journal of physiology, 1899, xxiii, supplement, p. 20), and at least the essential part of them by Frank (Sitz.-Ber. d. Ges. f. Morphol. u. Physiol., Munich, 1897, i), quoted from the Jahres-Ber. d. Physiol., 1898, vi, p. 64.

[2] HÜRTHLE: Archiv f. d. ges. Physiol., 1891, xlix, p. 89.

Whatever the explanation, it is undoubtedly a fact that at times when the accelerators and vagi are stimulated simultaneously the systole may be shortened as greatly as when the accelerators are stimulated alone, while the duration of diastole may be either unchanged or even slightly prolonged.[1] Such results as these do not prove, however, that these nerves are not purely antagonistic; they only show that when the effects upon the different phases of the cardiac cycle are considered, the strength of the currents were not comparable, and that in order to bring out the true relations it is necessary to consider the phases separately. If the accelerator and inhibitory nerves are stimulated with currents which when applied separately to the nerves cause changes in one or other of the phases which are comparable (*i. e.* neither stimulus must be a super-maximal one), the result is almost the exact arithmetical mean of the effect produced when the nerves are stimulated separately.

It is very easy to pass the limits of strengths of current which are comparable, and then the accelerator or inhibitory influence will preponderate in the one or the other phase, just as it does when the entire cardiac cycle is considered and one set of fibres is stimulated with a relatively much stronger current than the other.

The above considerations explain the cases in which one nerve gains the mastery in the one or the other phases of the heart's action.

The table on page 426, from one of my experiments, illustrates these points, and shows how perfect the antagonism of the accelerator and inhibitory nerves may be if care is taken to select stimuli of proper strength.

Thus when the two nerves were stimulated together the influence of the acclerators predominated during systole and that of the vagus during diastole, but in each case the effect of the one nerve was diminished by that of the other.

Mutual antagonism of the inhibitory and accelerator nerves in virtue of their tonic activity. — Since both the inhibitory and accelerator

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[1] Frank (*loc. cit.*) obtained similar results, but seems (I have not seen the original paper) to have interpreted them as indicating that the antagonism of the accelerators and inhibitory fibres is not perfect when their effect upon systole is considered.

	Duration of Systole.	Duration of Diastole.		Per cent.
Before stimulation	0.25″	0.37″		
Vagus stimulated alone . .	0.27″	0.61″	Systole prolonged . Diastole prolonged	8 65
Accelerators stimulated alone	0.18″	0.30″	Systole shortened . Diastole shortened	28 19
Both nerves stimulated . .	0.22″	0.45″	Systole shortened . Diastole prolonged	12 21

nerves are usually in tonic activity and are also antagonistic, it would seem to follow necessarily that the tonic activity of the one would limit the tonic activity of the other, so that the heart rate at any time (so far as it depends upon the cardiac nerves) is determined by the relative strength and number of the impulses reaching the heart from the central nervous system. In order to test this supposition it is desirable to determine the heart rate (1) when both nerves are intact and in tonic activity, (2) when the heart is under the influence of the accelerators alone, (3) when it is under the influence of the inhibitory nerves alone, and (4) when both nerves are divided. Such an experiment can be performed by suspending the activity of one of the nerves in such a manner that it can readily be restored again; this may easily be done by cooling the vagus. When the dog's vagus is cooled to about 0.° C., the power of the inhibitory fibres to conduct impulses is lost,[1] but it can readily be restored by warming the nerve; this method was employed in a number of experiments, of which the following is an example.

Experiment 153. Small bitch. Morphine and ether. Curare. Stellate ganglia carefully exposed. Vagi cooled by passing cold alcohol through metal tubes upon which the nerves lay.

Time. Hrs.	min.		Heart-beats in 10 seconds.	Time. Hrs.	min.		Heart-beats in 10 seconds.
10	45		17+	11	5		16½
		Vagi cooled.			12	Vagi cooled.	
	48		25		16		19+
	53		29+		17		20
		Vagi warmed.				Vagi cut.	
	55		16		18		20½
		Accelerators cut.					

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[1] Howell, Budgett, and Leonard: Journal of physiology, 1894, xvi, p. 304.

These results may be expressed in the following tabular form: —

Under the influence of both nerves the heart rate was	17+
Under the influence of the accelerator nerves the heart rate was	29+
Under the influence of the inhibitory nerves the heart rate was	16
After section of both nerves the heart rate was	20½

This experiment shows very clearly that the acceleration following section of the vagi is due in part to the tonic activity of the accelerators, but it shows also that it is not due entirely to this factor, for section of the vagi still caused some increase in the heart rate after section of the accelerators.

It follows from such experiments as the above that the extent of the acceleration following section of the vagi is determined not only by the condition of the cardio-inhibitory centre, but also by that of the accelerator centre. If the tonic activity of the accelerator centre is very great and that of the cardio-inhibitory centre small, section of the vagi will lead to but little increase in the heart rate. In the exceptional cases in which the accelerators are not in activity, the increase of the heart rate following section of the vagi is determined solely by the condition of the cardio-inhibitory centre. In some experiments the entire tonus of the inhibitory nerves seemed to be exerted in holding the accelerators in check; for while cooling of the vagi caused an acceleration of the heart when the accelerators were intact, after section of the latter nerves neither cooling nor section of the vagi caused any change in the heart rate.

In a similar manner the extent of the slowing of the heart following section of the accelerator nerves is determined not only by the condition of the accelerator centres, but also by that of the cardio-inhibitory centre.

That the normal heart rate is determined by the tonic activity of the cardiac nerves, can scarcely be doubted;[1] the varying effect of cutting all the nerves is strong evidence for this view. Thus after section of both the accelerator and inhibitory nerves one of three conditions results: (1) the heart rate is faster, (2) the heart rate is slower, or (3) the heart rate is the same as before the nerves were divided. Which of these three conditions results is determined by the relative strength of the impulses reaching the heart through the cardiac nerves. If the inhibitory impulses are the stronger, section of all the cardiac nerves causes the heart to beat more rapidly; if the accelerator impulses are the stronger, section of the nerves causes the heart rate to become slower.

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[1] Cf. v. CYON: Archiv f. d. ges. Physiol., 1898, lxx, p. 242.

In many of the experiments described above it is evident that the vagi restrained the activity of the accelerators;[1] and so, since fatigue and decrease in the irritability of the heart result from the action of the latter nerves, the vagi acted as a protection to the heart.

Moreover, there is evidence that, so far as the condition of the heart can be judged by its rate, stimulation of the vagus has a beneficial influence upon the mammalian heart, as Gaskell showed it to have in cold-blooded animals. Thus if the accelerators be intact and the peripheral end of one vagus (both vagi having been divided) be stimulated a number of times with currents of moderate intensity, the heart rate is frequently greater after than before the vagus was stimulated. This increase in the heart rate is not to be confounded with the acceleration which sometimes follows immediately after stimulation of the vagus, and which is due to the accelerator nerve fibres present in the vagus or vago-sympathetic trunk; the former is of much longer duration than the latter, and seems to be due to an improvement in the condition of the heart, as a result of which the impulses reaching this organ through the accelerator nerves become more effective. This effect of stimulating the vagus was well shown in an experiment upon a dog: the heart rate some time after section of the vagi was 33 in 10 seconds; the peripheral end of one vagus was stimulated with a weak, slowly interrupted induced current for 25 minutes, short intervals of rest alternating with longer periods of stimulation; after the stimulation the heart rate was 42 in 10 seconds, and it continued at this rate for some time. Such changes in the rate did not occur during periods of rest of equal duration.

Another illustration of the protective influence of the vagus over the heart is found in some experiments in which the vagus and accelerators were stimulated simultaneously for some time. Attention was called above to the fact that when the accelerators were thus stimulated it happened frequently that after the stimulation the rate of beat was considerably less than it was before the stimulation, and this was considered to be due to fatigue in the heart. If, however, the vagus was stimulated at the same time as the accelerators, the decrease in the rate after the stimulation ceased was, as a rule, much less, or did not occur at all. In other experiments, instead of stimulating the two nerves simultaneously the effect of stimulating the accelerators after a period of rest was compared with the effect of stimulating them after an equally long period of stimulation of the vagus; not only was the maximum acceleration greater in the latter case, but it was of much longer duration. It should be added that in order to obtain this result the stimulation of the accelerators must follow that of the vagus immediately; if a short interval, even one of a few minutes, elapsed between the two stimulations, the result was the same as when the accelerators were stimulated after a period of rest.

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[1] In a similar manner the tonic activity of the accelerators checks the action of the vagi; if the latter are thrown into activity reflexly, or if the nerves be stimulated directly, the effect upon the heart is less when the accelerators are intact; thus the accelerators prevent excessive action of the vagi, and so a long continued lowering of the blood pressure which might be injurious to the functions of some of the organs. Of course, if the stimulation of the vagi is excessive, as in asphyxia, for example, the effect of the accelerators may be entirely overcome, just as the effect of the tonic activity of the vagi may be entirely overcome by a powerful stimulation of the accelerators.

Finally, in most of the experiments in which death resulted from stimulation of the accelerators the vagi had been divided.[1]

PART II.

REFLEX ACCELERATION OF THE HEART.

The cause of reflex acceleration. — Many text-books of physiology allude to the possibility of reflex acceleration of the heart being produced in two ways: (1) by diminution of the tonus of the vagus, and (2) by stimulation of the accelerator centres. Very few of those who have studied this subject, however, speak of the former possibility; usually all cases of reflex acceleration are referred to stimulation of the accelerator nerve. This attitude is well illustrated by two of the most elaborate articles dealing with the innervation of the mammalian heart which have appeared within recent years — that of Roy and Adami,[1] and of v. Cyon.[2]

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[1] Death of the heart also resulted sometimes from the intravenous injection of hot normal saline solution or of Ringer solution, and it seemed to occur much more frequently in those animals in which the vagi had been divided. One experiment of this kind was especially interesting. Before the vagi were divided a considerable quantity of normal saline solution was slowly injected into the femoral vein from a fountain syringe; the temperature of the solution in the syringe was 53° C., but it was probably several degrees colder when it entered the vein. The injection increased the rate of beat, but not markedly (from 26½ to 34½ beats in 10 seconds), and no bad effects were produced; on the contrary, the condition of the circulation seemed to be decidedly improved. The vagi were divided, and some time afterwards a much smaller amount of the same solution was injected. The temperature of the liquid in the syringe was now but 50° C.; the heart-beats, however, were increased to 47 in 10 seconds; soon they could not be counted; the blood pressure fell and the dog died.

Roy and Adami describe reflex acceleration resulting from the stimulating of sensory nerves, and attribute it to a stimulation of the accelerator nerves; in fact, when studying the effect of these nerves upon the heart, these authors stimulated, as a rule, a sensory nerve instead of the accelerators directly, as they considered the reflex acceleration obtained in this manner to be equivalent to the effect of stimulating the nerves themselves.

Von Cyon describes reflex acceleration following the stimulation of a number of afferent nerves, and seems to ascribe it in all cases to a stimulation of the accelerators. Thus stimulation of a third root of the depressor,[3] which he has found in certain animals of different species, causes a reflex acceleration, and v. Cyon thinks that this shows that the nerve fibres of this root are connected in a special manner with the accelerator centre. It is worthy of note, however, that v. Cyon emphasizes the fact that in these experiments the vagi were intact, and in at least one experiment the vagus centre seemed to be in a condition of exaggerated activity as a result of the morphine used as an anæsthetic; hence there is no evidence that in these experiments the reflex acceleration was not due to a diminution of the tonic activity of the vagi. Von Cyon also describes an experiment in which stimulation of the central end of the superior laryngeal caused reflex acceleration;[4] but here also the vagi were intact and in tonic activity, as their subsequent section showed. Moreover, the increase in the heart rate from stimulating the superior laryngeal was far less than that following division of the vagi.

Careful examination of the accounts of various other experiments on this subject shows that in most of the cases in which reflex acceleration has been described the vagi were intact, and there is frequently evidence to show that they were in a condition of tonic activity. In the description of other experiments nothing is said as to whether the vagi were intact or not, while in a few the statement is made that reflex acceleration occurred after division of the vagi. Almost every statement of the latter kind with which I am acquainted is, however, open to criticism. Thus in some of the older experiments not only was the acceleration very slight, but it was accompanied in many instances by changes in the blood pressure sufficiently great to account for the changes in the heart rate.

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[1] ROY and ADAMI: Philosophical transactions, 1892, 183 B, p. 254.

[2] VON CYON: Archiv f. d. ges. Physiol., 1898, lxx, p. 126.

[3] This "third root" of the depressor springs from the superior cervical ganglion or from the cervical sympathetic. I may mention in this connection that Dr. Harrington and I found a small nerve in a calf lying very near the vagus and ending in the superior cervical ganglion; stimulation of this nerve caused a marked fall of blood pressure and a slight slowing of the heart. Thus this nerve corresponded. physiologically, to the depressor.

[4] VON CYON: *op. cit.*, p. 149, Table III.

Reflex acceleration has been described as resulting from stimulation of the depressor after section of the vagi; Bayliss,[1] for example, describes such an experiment, and reproduces a tracing showing the acceleration. An examination of this tracing shows that the acceleration is very different from that observed when the accelerators are stimulated directly, and arouses the suspicion that it is not a case of true reflex acceleration at all. When the accelerator nerves are stimulated directly, there is a long latent period, and the acceleration is developed slowly; after the stimulation ceases the heart returns slowly to its previous rate. In this tracing the heart rate remained unchanged during the first two or three seconds of stimulation; then it suddenly doubled, and the heart continued at this greater rate until after the stimulation had ended, when it suddenly returned to its previous rate, *i. e.* to just one half the accelerated rate. An examination of the heart in such cases as the above, in which the rate is slowed suddenly to one half the previous rate, usually shows that this is due to the failure of the ventricles to follow one half of the auricular beats; when, on the contrary, the heart rate is suddenly doubled, the ventricles are found to be responding to all of the auricular beats. In many animals this lack of co-ordination in the beats of the auricles and ventricles is made to disappear by influences causing slight changes in the blood pressure or respiration, by movements of the animal, by slightly pulling the vagi, etc., and it is possible that some such changes as these were the cause of the acceleration in Bayliss's experiment. On the other hand it must be remembered that stimulation of the accelerators increases the ease with which impulses are conducted from auricle to ventricle, and it is possible that in the above experiment the doubling of the heart rate was after all due to a reflex stimulation of the accelerators acting in this manner; still, I know of no experiments in which direct stimulation of the accelerators produced this effect without at the same time causing an increase in the rate of the entire heart.

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[1] BAYLISS: Journal of physiology, 1893, xiv, p. 313.

An experiment by Barbèra very similar to the one described by Bayliss is brought forward by v. Cyon[1] as evidence that reflex acceleration may under some circumstances be produced by stimulation of the central end of the cervical sympathetic. In this experiment, in which the vagi had been cut, the injection of a solution of sodium phosphate had caused a decrease of the heart rate to one half the previous rate; stimulation of the cervical sympathetic, as is shown by the curve published, caused the heart rate to increase from 10 to 20 beats in 5 seconds;[2] the latter was practically the rate at which the heart was beating before the injection of sodium phosphate. Apparently the heart continued to beat at this rate for some time after the stimulation ended. Evidently this experiment is open to the same criticism as the one discussed above, and it hardly can be regarded as a satisfactory case of true reflex acceleration.

Barbèra[3] describes the effect of stimulating the depressor after section of the vagi in the experiment cited by Cyon, just mentioned. He does not publish the curve, or give many details of the stimulation, but merely states that the heart rate increased as a result of stimulating the depressor from 240 to about 270 beats per minute, while the blood pressure fell from 103 to 86 mm. This may be a case of true reflex acceleration; but it may fairly be asked if in an animal in which, apparently as a result of repeated injections of sodium phosphate, the heart rate was as easily affected, as numerous statements show it to have been in this one, the fall of blood pressure may not have been the cause of this acceleration of the heart. I may add, moreover, that I know of no experiment in which the evidence for the occurrence of reflex acceleration after section of the vagi is stronger than in this one.

Attention was called above to the fact that very few of the authors who speak of reflex acceleration take into consideration the possibility of its being caused by diminution of the tonus of the vagi; in fact I know of but one paper (by MacWilliam) dealing directly with this subject. Before speaking of MacWilliam's work a few words may be said about some experiments of other physiologists bearing upon this question.

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[1] VON CYON: *op. cit.*, pp. 202-203.

[2] It may be that in some of these cases the doubling of the rate of the ventricle was only apparent, one strong beat being followed by a weaker beat; the result being that the two beats were recorded by the mercury manometer as one. However these changes are produced, they are evidently not satisfactory evidence of a reflex stimulation of the accelerators.

[3] BARBÈRA: Archiv f. d. ges. Physiol., 1897, lxviii, p. 444.

Schmiedeberg,[1] in his classical paper on the accelerator nerves of the dog, describes two experiments in which reflex acceleration was obtained by stimulating the central end of one of the limbs of the annulus of Vieussens. Schmiedeberg does not state whether the vagi were cut or not, but I infer that they were not, and from the fact that the heart rate increased later in the experiment I infer that they were in a condition of tonic activity. Schmiedeberg calls attention to the fact that in one of these two experiments the course of the acceleration was different from that resulting from direct stimulation of the accelerators, in that the after-effect was very short, and he expresses a doubt as to whether the acceleration was due to a reflex stimulation of the accelerator nerves; he does not suggest, however, that it may have been due to a diminution of the vagus tonicity.

Asp[2] made a number of experiments on the effect upon the heart rate of stimulating sensory nerves both before and after section of the vagi; he also made four experiments in which the accelerators were cut. Asp observed slight acceleration upon stimulating sensory nerves after the vagi were cut; the effect upon the blood pressure was variable. In two of the four experiments in which the accelerator nerves had been cut slight reflex acceleration occurred; in the two other it did not occur, although it had been obtained in all four experiments before the accelerators were cut. Asp was endeavoring to determine whether reflex acceleration is due to a stimulation of the accelerator nerves or, as he puts it, to "a constriction of the arteries of the brain, caused by a stimulation of the sensory nerve, by which the pressure on the vagus centres was reduced and the heart became more rapid." Neither Asp nor Schmiedeberg seems to have considered the possibility of the reflex inhibition of the cardio-inhibitory centre.

These experiments of Asp are sometimes cited as evidence of reflex stimulation of the accelerator nerves, and Asp himself was inclined to interpret them in this manner, although he states distinctly that he did not consider the question closed. The reflex acceleration was not marked; blood pressure changes could not be excluded, and finally, as Asp points out, the heart was irregular and the effect of stimulating sensory nerves very uncertain.

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[1] SCHMIEDEBERG: Sitz.-Ber. d. sächs. Gesell. d. Wiss., math.-phys. Cl., 1871, p. 152.

[2] ASP: Sitz.-Ber. d. sächs. Gesell. d. Wiss., math.-phys. Cl., 1867, p. 188.

Knoll[1] found that compression of the heart, either by the finger or by inflating the pericardium with air, caused an acceleration of the heart, and he showed that this acceleration was due to a diminution of the tonicity of the vagi.

MacWilliam[2] is apparently the only author who has given careful attention to the question as to the manner in which reflex acceleration is brought about when an ordinary sensory nerve is stimulated, but he has published only a preliminary paper containing few details. MacWilliam compared the latent period of direct and of reflex acceleration, and reached the conclusion that the latent period of the latter is too short for the acceleration to be referred to a stimulation of the accelerator nerves; he thinks it is due to a diminution of the tonus of the vagi. The effect upon the heart rate of stimulating the sensory nerves before and after section of the vagi, and of stimulating the accelerators, was also studied by MacWilliam, with the result that reflex acceleration was obtained after the accelerators were cut if the vagi were intact, but not after the vagi were cut, although the accelerators were intact. From these experiments MacWilliam drew the conclusion that, ordinarily, reflex acceleration is due to a diminution of the tonus of the vagi.

The afferent nerves by which reflex acceleration is produced. — The nature of the afferent nerve fibres by which reflex acceleration and reflex slowing of the heart are produced has received but little attention. Tigerstedt,[3] after discussing the various experiments on this subject, states what may perhaps be regarded as the current view of physiologists, namely, that both the accelerator and inhibitory nerves can be thrown into reflex activity by the stimulation of almost all afferent nerves.

There is evidence, however, that the various sensory nerves differ in their effect upon the heart; some for example cause as a rule reflex acceleration, others, reflex slowing. To the latter class, as Tigerstedt pointed out, belongs the trigeminus; stimulation of this nerve seems always to cause reflex slowing, if it has any effect at all upon the heart. Asp[1] states that, as a rule, he observed acceleration to follow stimulation of the central end of muscular branches of nerves; but in some experiments reflex slowing occurred. Tengwell,[2] however, found the stimulation of muscular nerves had but little effect upon the heart rate. Roy and Adami[3] found reflex acceleration usually resulted from stimulation of the sciatic nerve; sometimes the acceleration was followed by a slowing of the heart. The latter nearly always occurred when the splanchnic nerve was stimulated. Schmiedeberg[4] observed in two of his experiments that stimulation of the central end of one limb of the annulus of Vieussens caused reflex acceleration, whereas stimulation of the other limb caused reflex slowing; he suggests that there are several varieties of afferent nerve fibres, some of which cause reflex acceleration and some reflex slowing.

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[1] KNOLL: Lotos, neue Folge, 1881, ii, p. 14.

[2] MACWILLIAM: Proceedings of the royal society, London, 1893, liii, p. 464. It is but fair to myself to state that most of my experiments were performed before I learned of MacWilliam's paper; in fact, the greater part of this section of my paper was written a number of years ago, and originally included in a thesis presented to the Johns Hopkins University for the degree of doctor of philosophy.

[3] TIGERSTEDT: Lehrbuch der Physiologie des Kreislaufes, 1893, p. 289.

Statements and speculations as to the nature of the afferent nerve fibres concerned in reflex acceleration, however, have only a secondary interest, so long as the question of the manner in which this acceleration is caused is left undecided; for if it should be shown that reflex acceleration is in reality due to an inhibition of the cardio-inhibitory centre, the effect of stimulating various nerves might be determined solely by the condition of this centre at the time of stimulation.

ON THE MANNER IN WHICH REFLEX ACCELERATION IS PRODUCED.

The problem whether the reflex acceleration resulting from the stimulation of sensory nerves is caused by a diminution of the tonicity of the vagi, or by an increased action of the accelerators, was approached from three standpoints: (1) the details of some of the events occurring in the heart when it was thrown into a condition of acceleration in various ways were investigated, (2) the accelerators were cut and sensory nerves stimulated, and (3) the vagi were divided and sensory nerves stimulated.

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[1] ASP: *op. cit.*, p. 183. The statement of Asp that mechanical stimulation of the sciatic plexus causes a reflex slowing of the heart, whereas electrical stimulation causes an acceleration. is often quoted. I can find but one experiment of this kind described in Asp's paper (p. 182), and this is most unsatisfactory on account of the great differences produced in the blood pressure in the two cases; with mechanical stimulation the blood pressure rose 106 mm., while with electrical stimulation there was a rise of but 37 mm. Some of Asp's experiments upon the effect of stimulating the lumbar cord are open to a similar criticism; thus he compares the effects of two stimuli (one mechanical and the other electrical) upon the heart rate, although in the one case the blood pressure rose to 100 mm. and in the other to 174 mm. Some of these experiments will be referred to again.

[2] TENGWELL: Skandinavisches Archiv f. Physiol., 1895, vi, p. 230.

[3] ROY and ADAMI: Philosophical transactions, 1892, 183 B, p. 258.

[4] SCHMIEDEBERG: Ber. d. sächs. Gesell. d. Wiss., math.-phys. Cl., 1870, p. 152.

The duration of systole and diastole and the latent period when the heart rate is increased in various ways. — The duration of systole and diastole, and the latent period of acceleration, were determined when the heart rate was increased by the stimulation of a sensory nerve, and these results were compared with those obtained by cutting the vagi and by stimulating the accelerators directly. If the acceleration occurring in the former case is due to a diminution of the tonic activity of the vagi, we should expect to find the course of the acceleration similar to that resulting from section of the vagi, rather than to that observed when the accelerators are stimulated directly; the former is, as a rule, the case, as the following experiments show.

The duration of systole and diastole, and of the latent period, was determined by means of Hürthle's manometer, in the manner described in the first part of this paper. At times the dicrotic wave on the curve of carotid pressure became so indistinct that it was impossible to determine accurately the duration of systole; such tracings could be used, however, for determining the latent period.

The results obtained from the various experiments were so uniform that only one or two experiments of each class need be described.

Section of the vagi. — The following experiment shows the usual effect upon the heart-beat of cutting the vagi when these are in a condition of tonic activity.

Experiment A. Very small dog. Morphine and ether. Left carotïd connected with Hürthle's manometer. Left vagus had been cut.

Time.			Duration in seconds of		Time.			Duration in seconds of	
Hrs.	min.		Systole.	Diastole.	Hrs.	min.	sec.	Systole.	Diastole.
3	50		0 175	0.495				0.165–	—
			0.165	0 495	3	50		—	—
		R. vagus cut.	0.175	0.365+				0 155+	0.260
			0.165	0.365+				—	0.260
			0.170–	0.330				0.150–	—
			0.165	0.305				0.145	—
			—[1]	—				—	—
			—	0.280+			10	0.140	0 255
			0.165–	—			12	0.130+	0.255
			—	—			14	0.120	0.255

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[1] In this and subsequent tables the dash (—) means that the curves were not counted out in full; the labor of counting these tracings to hundredths of seconds is very great.

These results are shown in the plotted curve of Fig. 6 better than in the table; the ordinates represent the duration of systole and diastole in 0.05 of a second, the abscissæ five heart-beats. In Fig. 7 a small part of the original tracing is reproduced.

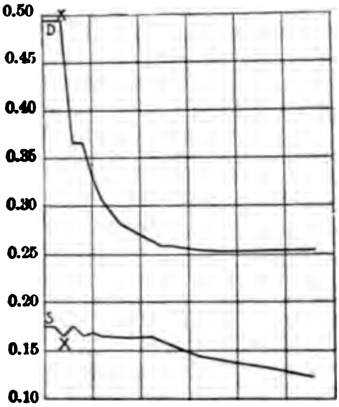

FIGURE 6. Experiment A. Section of right vagus. The ordinates represent the duration of systole, *S*, and of diastole, *D*, in 0.05 seconds; the abscissæ, 5 heart-beats.

The chief effects upon the heart rate of cutting the vagi, as shown by the above experiment, are (1) the very sudden and great decrease in the duration of diastole, and (2) the much more slowly developed and relatively less marked effect upon the systole. The shortening of diastole began in the heart-beat during which the vagus was cut, and had reached almost the maximum before the shortening of systole began. These results are in accord with the well-known fact that it is the diastole which is most easily and quickly affected when the heart rate is altered, and that the influence of the vagus upon diastole is greater than its influence upon the systole.

Results very similar to the above are obtained when the heart returns to its normal rate after it has been slowed by stimulation of the peripheral end of the vagus or when it has been slowed reflexly.

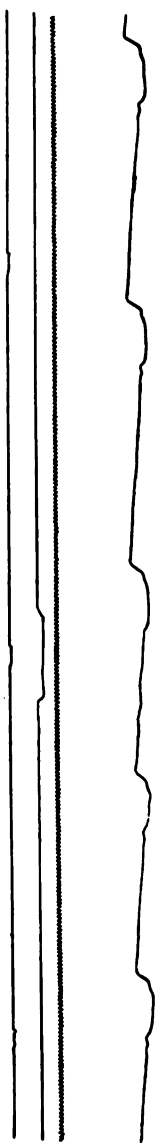

FIGURE 7. Two thirds the original size. Experiment A. Part of record taken with Hürthle's manometer to show effect of cutting the right vagus. Time in intervals of 0.01 and 1 second intervals. To be read from left to right.

Stimulation of the accelerators.—An experiment was described in the first part of this paper (p. 402) showing the effect upon the duration of the systole and diastole of cutting the accelerator nerves; the following table and curve (Fig. 8) show the effect of stimulating the accelerators in this experiment.

Time. Hrs.	min.	Duration of Systole.	Duration of Diastole.	Heart-beats in 10 seconds.
2	3	0.285	0.310	16½
		0.300+	0.325	
R. ann. stim.; coil 10 cm.				
		0.285	0.305	
		0.265	0.330	
		0.300	0.330	
		0.280	0.305	21½
		0.265	0.265	
		0.240+	0.275	
		0.235	0.260	
		0.215+	0.245	
		—	—	
		0.230	0.240–	
		—	—	
		0.195	0.225	
		—	—	
		—	—	
		0.180	0.220+	
		—	—	
		0.175	0.210+	
		—	—	
		—	—	
		—	—	
		0.160	0.215+	
		0.155	0.200	
Stim. off annulus.				
		0.160	0.190+	(Every 5th beat counted.)
		0.145+	0.205	28+
		0.140+	0.210+	
		0.145+	0.205+	
		0.140	0.220	

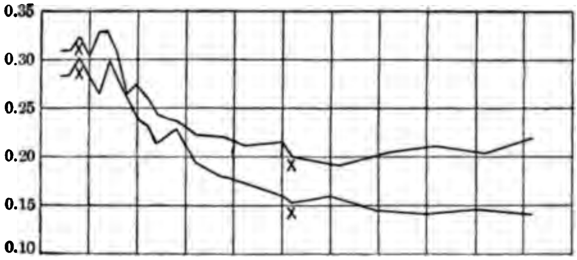

FIGURE 8. Experiment C. Stimulation of the right annulus (x to x). The ordinates represent the duration of systole, *S*, and of diastole, *D*, in 0.05 seconds; the abscissæ, 5 heart-beats.

The exceptionally long duration of the systole in this experiment was caused by the section of the accelerators.

This experiment shows that stimulation of the accelerators causes a shortening of systole as well as of diastole, and that the latent period of each is very long; in fact, the maximum shortening occurred after the cessation of the stimulation.

Curves very similar to the above are obtained when the accelerators are stimulated during a long stimulation of the vagi, by which the heart is slowed. Occasionally, however, when the heart rate is very slow, either in consequence of the stimulation of the vagi,[1] or of the tonic activity of these nerves, or from other causes, and the stimulus applied to the accelerators is very strong, the latent period is much shorter than in the above experiment,[2] and the diastole is much shortened before the shortening of the systole begins. In such cases the curve is intermediate in form between those resulting from section of the vagi and those usually following stimulation of the accelerators.

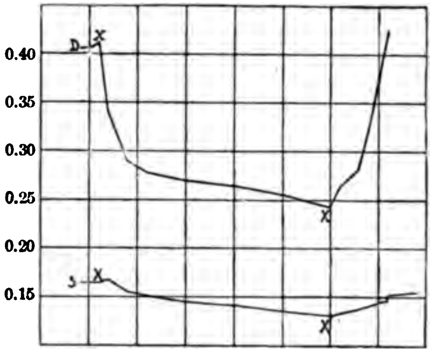

FIGURE 9. Experiment E. Stimulation of saphenous. (See above table.) The ordinates represent the duration of diastole, *D*, and of systole, *S*, in 0.05 seconds; the abscissæ represent 5 heart-beats.

Acceleration of the heart resulting from stimulation of a sensory nerve. — The following experiment illustrates the effect upon the heart rate of stimulating a sensory nerve.

Experiment E. Medium-sized dog. Morphine and ether; curare. The following table and curves (Figs. 9 and 10) show the effect upon the heart of stimulating the saphenous nerve.

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[1] François-Franck (Travaux du laboratoire de Marey, 1878–79, p. 80) states that the latent period is prolonged when the heart is under the influence of the vagus; I have found just the opposite to be the case. There is, moreover, the following difference: when the vagi have been divided, or when they are not in tonic activity, the latent period of the accelerators is found to be long in both systole and diastole, and the shortening of one does not begin before that of the other. If, however, the vagi are in activity, stimulation of the accelerators may cause a marked shortening of diastole before the duration of systole is at all affected. When, on the other hand, the vagus is stimulated, the diastole is always prolonged before the systole, *i. e.* the latent period of stimulation of the vagus applies especially to the systole.

[2] Hürthle (Archiv f. d. ges. Physiol., 1891, xlix, p. 89) describes an experiment on a very slowly beating heart in which the second diastole was shortened; I never have observed a shortening of any diastole before the fourth as a result of stimulating the vagus.

Time. Hrs. min.	Duration in seconds of Systole.	Diastole.
11 23	0.175	0.415
	0.175	0.420
R. saphenous stimulated for 10 secs.; coil 11 cm.		
	0.180–	0.350
	—	—
	0.165	0.300
	—	—
	0.160	0.290
	—	—
	—	—
	—	—
	0.155+	0.280
	—	—
	—	—
	—	—
	—	—
	0.150	0.275
	—	—
	—	—
	—	—
	—	—
	0.145	0.265
	—	—
	—	—
	—	—
	—	—
	0.140	0.255
Stimulus off saphenous.		
	0.140+	0.270+
	—	—
	0.145+	0.290+
	—	0.330+
	0.150+	—
	0.160	0 430+
	—	—
	—	—
	0.165	0.505+

Examination of the above table and curves shows that the shortening of the diastole is very marked and the latent period very short; the effect upon the systole is less marked and is more slowly developed. The heart rate also returned very quickly to the normal.

When the effects upon the heart rate of stimulating a sensory nerve are compared with those described above, resulting from cutting the vagi and from stimulating the accelerators, it is very evident that they resemble the former much more closely than they do the latter. When the heart is accelerated by section of the vagi, or by the stimulation of a sensory nerve, the diastole is shortened relatively much more than the systole, and the latent period is very short. When, however, the accelerators are stimulated directly, the systole as well as the diastole is much shortened, and the latent period is very long. Moreover, the after-effect upon the heart rate of stimulating the accelerators directly is often very different from that occurring in reflex acceleration; in the former case the heart continues beating at a rapid rate for some time, whereas in the latter it often returns very quickly to its previous rate or is slowed.

As the results described above are by no means exceptional, but are the rule, I think they can be regarded as strong evidence for the view that in reflex acceleration of the heart diminution of the tonic activity of the vagi plays the chief rôle.

These results are of a special interest, since they suggest that under certain circumstances the cause of any sudden increase in the heart rate, *i. e.* as to whether the increase is due to stimulation of the accelerators or to a diminution of the tonic activity of the vagi, can be determined from the cardiogram of the intact animal or of man.

While in most cases the curves of direct and of reflex acceleration differ in the manner described above, there are exceptional cases in which the difference is not well marked. In some cases of reflex acceleration, for example, the latent period is comparatively long; this occurs most frequently when the heart rate is already rapid and the acceleration relatively small. On the other hand, the latent period when the accelerators are stimulated may be very short; as already described, this occurs when the heart is beating very slowly. Further, the difference mentioned above in the effect upon systole and diastole, in the two cases, is not always marked. Even in these exceptional cases, however, the subsequent course of acceleration usually differs; the maximum shortening is reached much more quickly in reflex than in direct acceleration.

Reflex acceleration after section of the accelerators. — Such an experiment as the following shows that marked reflex acceleration of the heart may occur after the principal nerves containing accelerator fibres have been divided.

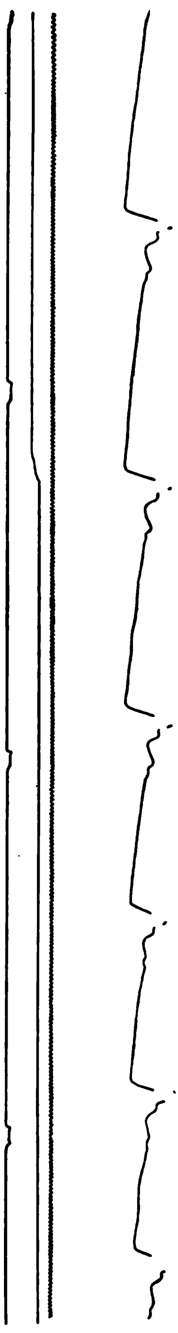

FIGURE 10. One half the original size. Experiment E. Part of record taken with the Hürthle manometer to show effect of stimulating saphenous nerve; secondary coil 7 cm. Time in 0.01 and 1.0 second intervals. To be read from left to right.

Experiment 139. Small dog. Morphine and ether. Stellate ganglia and their branches exposed. Blood pressure from left femoral artery.

Time. Hrs.	min.		Heart-beats in 10 seconds.
1	8		14–
		R. saphenous stim. for 10 sec.; coil 10 cm.	22–
			13½
			12+
			11½+
1 to 1	30 34	Accel. nerves cut.	
1	39		14½
		R. saphenous stim. for 10 sec.; coil 10 cm.	19½
			11½
			10

Time. Hrs.	min.		Heart-beats in 10 seconds
			—
			10½
	40½		10½
		R. saphenous stim. for 10 sec.; coil 14.	18½
			10½–
			10
			9½+
			10½
	51		11
		R. saphenous stim. for 10 sec.; coil 16 cm.	17+
			11½
			10½

Section of the vagi caused the heart rate to increase to 26 beats in 10 seconds.

The accompanying curve (Fig. 11) shows the effect upon the heart rate of one stimulation of the saphenous nerve in the above experiment after the accelerators were cut. A glance at this curve shows it to be very different from those obtained when the accelerators are stimulated directly; in the latter case the latent period is much longer, the maximum acceleration is much more slowly reached, and the heart rate returns much more slowly to its previous rate. The reflex acceleration in this experiment was slightly greater before than after the accelerators were cut; this feature, which frequently

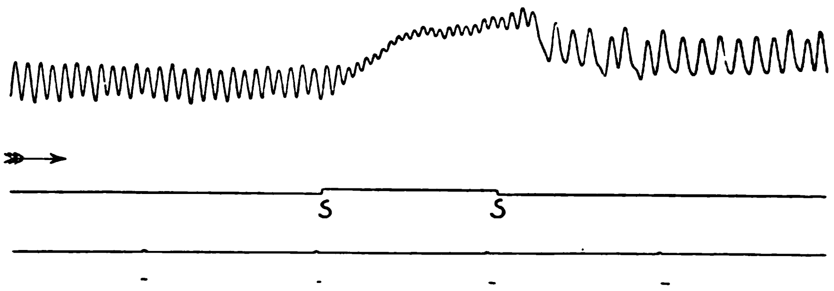

FIGURE 11. Two fifths the original size. Experiment 139. Acceleration of the heart resulting from stimulation of the saphenous nerve, *S–S*, after section of the accelerator nerves. Time in intervals of 10 seconds. Tracing to be read from left to right.

occurs, will be discussed below, but it evidently does not interfere in the least with the conclusion that in reflex acceleration diminution of the tonicity of the vagi plays the chief rôle.

Results similar to the above were secured in a large number of experiments. In fact whenever reflex acceleration was obtained with the accelerators intact, it was obtained also after these nerves had been divided, provided the tonicity of the vagus centre was not destroyed by the operation. The operation necessary to expose the accelerators and the section of these nerves tend to reduce or to abolish permanently the vagus tonicity; if this occurs, no reflex acceleration takes place.[1] In order to get the best results it is well to proceed as in the above experiment; the stellate ganglia should be exposed with as little operating as possible, and then some time allowed for the animal to recover. The temperature is prevented from falling by keeping the animal on a zinc box filled with warm water, and the wounds are carefully covered with towels wet with warm saline solution. Finally the nerves are cut with a pair of very sharp scissors, and pulling or crushing of the nerves carefully avoided. All cases in which reflex acceleration occurred before but not after section of the accelerators could be explained by the loss of vagus tonicity resulting from the operation. On the other hand, I had never observed reflex acceleration to occur except when there was clear evidence that the vagi were in tonic activity.[2]

Another point of interest in this connection (and the one which first led me to suspect that reflex acceleration is due to a diminution of the vagus tonicity) is that the rate reached by the heart during reflex acceleration is never greater than that following the section of the vagi; it may reach this rate, but never exceeds it.

Certain objections may be raised to the conclusion that in such experiments as the above the acceleration was due to a reflex diminution of the tonic activity of the vagi. Thus the acceleration might be attributed to the rise of blood pressure which so often occurs at the same time. It is not at all uncommon, especially when the heart is beating very slowly, for stimulation of a sensory nerve to cause a rise of blood pressure and a considerable acceleration of the heart even after all the cardiac nerves are divided;[1] the same result may follow stimulation of the peripheral end of the splanchnic, both when the cardiac nerves are intact and when they have been divided. The course of the acceleration occurring in such cases as these, however, is very different from that which occurs when a sensory nerve is stimulated and the vagi are intact. In the latter case the acceleration begins with considerable suddenness; the second or third beat may be shortened and a very distinct acceleration occur before the blood pressure has begun to rise; in the former case the acceleration begins very slowly; I have never observed it to begin until after the nerve had been stimulated for at least 10 seconds, and the blood pressure had risen very considerably.

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[1] It is not improbable that the absence of reflex acceleration in the two experiments of Asp referred to above (p. 433), in which the stellate and inferior cervical ganglia had been removed, was due to the loss of tonicity of the vagi resulting from the operation.

[2] Reflex acceleration also occurs in animals in which subsequent section and stimulation of the accelerator nerves show them to have been in a condition of maximum acceleration; the reflex acceleration in such cases is obviously due to an inhibition of the vagus centre.

Moreover, reflex acceleration frequently takes place when the change in the blood pressure is insignificant.

Another possible cause of the acceleration occurring after the stellate ganglia and their branches have been extirpated remains to be considered, viz. a reflex stimulation of accelerator fibres which pass to the heart in the vagus or vago-sympathetic trunk. As is well known, the slowing of the heart caused by stimulation of the peripheral end of the vagus or of the vago-sympathetic in the dog is sometimes followed by an acceleration; if atropine has been given, acceleration alone may result. This acceleration at present is usually explained by supposing that there are certain accelerator fibres which reach the heart by this route, and the possibility of reflex acceleration being produced by means of such fibres must be considered. Certain considerations, however, make it very improbable that these fibres play any important part in the production of reflex acceleration. In the first place, it is rather exceptional to find any evidence for the existence of accelerator fibres in the vago-sympathetic of the dog; reflex acceleration is very frequently obtained after removal of the stellate ganglia in animals in which no acceleration is observed when the vago-sympathetics are stimulated either before or after the administration of atropine. When stimulation of the vagus does cause acceleration after atropine, the curve closely resembles that observed when the other accelerators are stimulated, and is not at all like that obtained in reflex acceleration. Moreover, the acceleration caused by the direct stimulation of these nerves is seldom so great as that which is often obtained reflexly by the stimulation of sensory nerves.

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[1] The acceleration observed in such cases may not be due to the rise of blood pressure, but to the warm blood which is forced suddenly into the heart from the abdominal viscera. Cf. MARTIN: Physiological papers, 1895, p. 18.

Stimulation of sensory nerves after section of the vagi. — The question, Does reflect acceleration occur after section of the vagi? may now be discussed. It may be said at once that in a large number of experiments stimulation of sensory nerves caused either no acceleration at all after section of the vagi, or the acceleration was so slight that it could be referred probably to changes in blood pressure; in no case was there evidence that the slight acceleration was due to stimulation of the accelerator nerves, for such changes in the heart rate occurred after these nerves as well as the vagi had been cut. No reflex acceleration took place after the vagi had been cut in cases in which all the conditions seemed very favorable, *i. e.* in cases in which marked reflex acceleration had been observed before the vagi were cut, and in which, as subsequent examination showed, the accelerators were not in a condition of maximum activity and were very irritable.

The question may be raised, if after the vagi were cut the heart was not already beating at so rapid a rate that the conditions were not favorable for the occurrence of reflex acceleration, although the accelerator centres may have been irritable. This rapid rate of the heart, however, could not account for the entire absence of reflex acceleration, for the maximum rate to which the heart can be accelerated is independent of the rate at which the heart beats before stimulation,[1] and it is very rare for the heart to be in a condition of maximum acceleration after section of the vagi. Still, if the heart was beating slowly before reflex acceleration occurred, the relative increase would be greater, and hence more striking. Accordingly, in a number of experiments in which the heart rate had increased as a result of cutting the vagi, the peripheral end of one or of both of these nerves was stimulated with a current just sufficient to bring the heart back to the rate at which it was beating before the nerves were cut. Stimulation of sensory nerves in such cases never caused any acceleration, although direct stimulation of the accelerators caused a great increase in the rate.

These experiments are of interest in connection with the work of Roy and Adami. These authors agree with me as to the absence of reflex acceleration after section of the vagi,[1] but give an entirely different explanation. Roy and Adami explain reflex (and also direct) acceleration of the heart as due to a diminution of the vagus tonus *in the heart* resulting from the action of the accelerator nerves; after section of the vagi, reflex or direct acceleration of the heart is impossible, since there is no vagus tonus in the heart to overcome. If this explanation were correct, it is impossible to see why reflex acceleration does not occur when the effect of the vagi upon the heart has been restored by the artificial stimulation of these nerves.

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[1] Bowditch: Ber. d. sächs. Gesell. d. Wiss., math.-naturw. Cl., 1871, p. 266.

A few experiments on the effect upon the heart rate of stimulating the cerebral cortex may be referred to here. The suggestion that the accelerator nerves may be thrown into activity by processes originating in the cerebrum is frequently made; hence it seemed possible that stimulation of the cerebral cortex might yield interesting results. Accordingly in a number of experiments upon dogs the motor areas and various parts of the frontal and occipital lobes were stimulated with the faradic current. Acceleration of the heart frequently occurred when the vagi were intact and in tonic activity, but no acceleration was obtained after section of the vagi, either when the heart was beating rapidly, or when it was slowed by stimulating the peripheral end of the vagus.

Influence of the tonic activity of the accelerators upon reflex acceleration. — Although, as has been shown above, reflex acceleration is due to a diminution of the tonus of the vagi, and occurs after the accelerator nerves have been divided, yet these nerves, if they are in a condition of tonic activity, exert a modifying influence upon the course of the acceleration. The extent of the reflex acceleration, like the increase in the heart rate following section of the vagi, is in fact determined by two factors, — the cutting off of the influence of the tonic activity of the vagi, which of itself keeps the heart beating slowly, and the removal of the check to the tonic activity of the accelerators.

This influence of the tonic activity of the accelerators is shown in three ways. In the first place, the rate to which the heart is accelerated is, as a rule, greater when these nerves are intact than when they have been divided. Secondly, the course of the acceleration is, or may be, somewhat different; there may be, to begin with, a very sudden and marked acceleration, due to the inhibition of the tonic inhibitory impulses, and then an acceleration more slowly developed, due to the accelerator nerves; whereas after section of the accelerators reflex acceleration usually reaches its maximum very quickly, and is often followed by a partial return to the normal while the stimulation continues. In the third place, the after-effect of the stimulation is often different: when the accelerators are intact, the rate may continue rapid for some time after the stimulation ceases, and only slowly return to the normal, or give way to a secondary slowing. If, however, the accelerators have been divided, the rate after stimulation of a sensory nerve usually returns quickly to the normal, or perhaps more frequently is followed by a reflex slowing.

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[1] Roy and Adami: Philosophical transactions, 1892, 183 B, p. 267.

All three of the above points are illustrated by the curves in Fig. 12; these results were obtained from an experiment upon a dog.

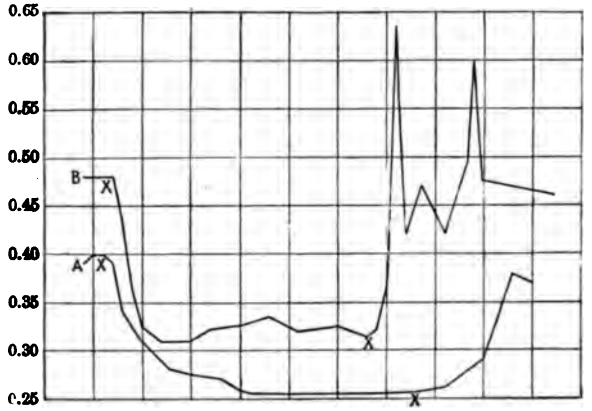

FIGURE 12. Experiment H. Stimulation of the saphenous, *A*, before, *B*, after, section of the accelerator nerves. The stimulation continued 10 seconds in each case; the strength of the stimulating current was also the same. Ordinates represent the duration of the heart-beat in 0.05 seconds; the abscissæ, 5 heart-beats.

Stimulation of the saphenous in this case, when both vagi and accelerators were intact, caused the heart rate to increase from 22½ to 31+ beats in 10 seconds. Section of the accelerators caused the heart rate to decrease to 19½ in 10 seconds; stimulation of the saphenous now with the same strength of current as above caused the heart rate to be increased to but 24½ beats in 10 seconds. Of course the objection may be made that the more rapid rate in the former case (*i. e.* when the heart rate increased to 31) was due in part to a reflex stimulation of the accelerators, and not exclusively to a diminution of the tonic activity of the vagi. That, however, the latter factor alone is sufficient to account for the acceleration is shown by the following fact: before the saphenous nerve was stimulated or the accelerators cut, the conductivity of the vagi was suspended by ether vapor with the result that the heart rate increased to 34 in 10 seconds.

The rate 31 therefore does not represent the maximum acceleration which might have resulted from inhibition of the inhibitory impulses. The vagi were afterward cut with the result that the heart rate increased to 29 beats in 10 seconds.

To summarize the results of this experiment and to show how completely the heart rate is determined at any given time by the interaction of the inhibitory and accelerator nerves, the following table may be given.

	Heart rate in 10 seconds.
Vagi and accelerators intact and in tonic activity	22
Activity of vagi suspended by ether vapor, the accelerators being intact	34
Accelerators cut, vagi intact and in tonic activity	19
After section of accelerators and vagi	29
Reflex acceleration, accelerators and vagi being intact	31
Reflex acceleration after section of the accelerators	24
Stimulation of accelerators (weak current), vagi being intact	27
Stimulation of accelerators (current as before), vagi cut	34½

If, returning to the course of reflex acceleration, we compare the two curves given in Fig. 12, the following differences will be observed. When the accelerators were intact and the saphenous nerve stimulated, there was first a sudden marked shortening of the duration of the heart-beat; this was followed by a longer period in which the heart rate was very slowly increased. After cessation of the stimulation the acceleration of the heart continued for some little time. When, on the other hand, the saphenous was stimulated after section of the accelerators, there was also a sudden increase in the heart rate; but this was soon followed by a slight slowing, and immediately after the stimulation there was a very marked slowing.

The curves given above are intended only to illustrate the differences which the section of the accelerators may make in a given experiment in the course of reflex acceleration; they are not intended to serve as types of the reflex acceleration occurring before and after section of the accelerators. No such types can be given, for the course of acceleration is seldom the same in any two experiments. Thus in one experiment the acceleration may be followed by reflex slowing, even during the stimulation of the sensory nerve, although the accelerators are intact and in tonic activity; whereas in another experiment reflex acceleration may continue long after the cessation of the stimulation, although the accelerators have been divided. In any one experiment, however, the section of the accelerators usually has some such effect as that described in the above case, *i. e.* the maximum acceleration is less and is more quickly developed and there is a greater tendency to a subsequent slowing.

The parts taken by the inhibitory and accelerator nerves in causing reflex changes of the heart rate find a striking analogy in the relation of the oculo-motor and sympathetic nerves in reflex movements of the pupils. Thus, according to Braunstein,[1] reflex dilatation of the pupil is due to an inhibition of the tonic activity of the oculo-motor centre, for it occurs after section of all the pupil-dilating fibres, if the oculo-motors are intact; on the other hand, no reflex dilatation of the pupil occurs after section of the oculo-motors although the pupil-dilating fibres are intact.[2] Moreover, just as the tonic activity of the accelerator nerves modifies the course of reflex acceleration of the heart, so the tonic activity of the pupil-dilating fibres modifies the course of the reflex dilatation of the pupil; after section of these fibres the dilatation occurs more slowly, as some of the "dilating force" is lost.

Some influences affecting the condition of the cardio-inhibitory centre. — With the exception of the respiratory centre there is perhaps no medullary centre in the cat and dog so easily influenced as that of the inhibitory nerves of the heart; Hering's experiments on rabbits show that this, probably, is true for these animals also.[3]

No attempt will here be made to discuss the influences affecting the condition of the cardio-inhibitory centre with any degree of fulness; only a few factors will be noted which it is necessary to take into consideration in connection with the question of reflex acceleration.

Some influences increase, others decrease, the tonic activity of the cardio-inhibitory centre; among the former may be mentioned high blood pressure and deficient respiration, and among drugs morphine and, apparently, small doses of curare. Morphine causes slowing of the heart not only by its direct action upon the cardio-inhibitory centre, but also through its action upon the respiration. In animals anæsthetized by means of cerebral pressure the vagi are usually found to be in a condition of marked tonic activity.

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[1] BRAUNSTEIN: Zur Lehre von der Innervation der Pupillenbewegung, p. 95; Wiesbaden, 1894.

[2] In man, also, no reflex movements of the pupil are obtained after complete paralysis of the oculo-motor.

The dilatation of the pupil resulting from stimulation of the cerebral cortex is also due, according to Braunstein (p. 116), to a diminution of the tonus of the oculo-motor centre.

[3] HERING, H. E.: Archiv f. d. ges. Physiol., 1895, lx, p. 429.

Ether and large doses of curare, and of chloroform, decrease or abolish the tonus of the vagus centre completely;[1] operations and loss of blood have the same effect, so that unless great care is taken in operating, the tonus of the vagi is completely lost.

Not only does the extent of the tonus of the vagi vary widely in different animals of the same species, but the susceptibility of the centre to influences which increase or decrease the tonus also varies in different individuals. In most dogs, for example, morphine causes a very slow heart beat, but in some it has almost no effect upon the heart rate, although given in very large doses.

The tonic activity of the vagi seems to be very slight in young animals, and to be very easily abolished in them.

Influence of the condition of the cardio-inhibitory centre upon cardiac reflexes. It is obvious that no inhibition of a centre can occur unless the centre is in a condition of activity; but the question arises, Is a centre more easily inhibited the greater the activity, or is it more easily inhibited when influences are at work which tend, of themselves, to weaken the activity? The results of my experiments upon the cardio-inhibitory centre indicate very clearly that the latter is the case; this was shown best, perhaps, in experiments on dogs narcotized with morphine. In such animals the cardio-inhibitory centre is in a condition of marked activity, and stimulation of a sensory nerve often has at first no effect; if, however, some drug which of itself tends to destroy the tonus of the centre is given, the stimulation of a sensory nerve may cause a marked acceleration of the heart.

Among the drugs which reduce the irritability of the cardio-inhibitory centre are ether and curare, as was mentioned above; if one of these is given to an animal under the influence of morphine, there may be an increase of the heart rate of short duration, and then the slow rate returns; stimulation of a sensory nerve now, however, may easily cause an inhibition of the centre and so an acceleration of the heart. Similar results are obtained in animals anæsthetized by cerebral pressure; in these animals the cardio-inhibitory centre is in a condition of strong activity, and stimulation of sensory nerves may have no effect upon the heart or may cause a slowing. If, however, ether or curare is given, — although no visible change is produced, — stimulation of a sensory nerve usually will cause an inhibition of the centre.

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[1] An irregular rhythm of the heart, probably due to the inhibitory nerves, is frequently made to disappear by the administration of ether or of curare, or by the stimulation of a sensory nerve, all of which cause a diminution of the vagus tonus.

If both vagi are intact and in tonic activity, stimulation of a sensory nerve may have no effect upon the heart rate; but if one vagus be cut, — although this of itself may cause no increase in the heart rate, — stimulation of the same sensory nerve may cause a marked acceleration.

Just the converse of the above holds good for reflex slowing of the heart. If the irritability of the cardio-inhibitory centre has been much reduced so that the heart is beating very rapidly, stimulation of a sensory nerve may have no effect upon it; if, however, the respiration is diminished, or the blood pressure increased in some manner, or intravenous injections of warm normal saline solutions be made, — influences which tend to stimulate the centre, — then stimulation of a sensory nerve may cause a marked slowing of the heart.

Such experiments as those briefly sketched above show that in order to obtain either reflex inhibition or reflex excitation of the cardio-inhibitory centre it is necessary that this centre be in a condition of unstable equilibrium; if the centre is in this condition, the result of stimulating a sensory nerve is determined in part (but only in part, as will be shown later) by the rate at which the heart is beating when the nerve is stimulated: if the heart is beating slowly, reflex acceleration results; if it is beating rapidly, reflex slowing occurs. Thus, in one of many experiments the heart rate was 24 in 10 seconds, and stimulation of the saphenous nerve caused the heart rate to decrease to 17½. A small amount of warm normal saline solution was injected into the femoral vein, as a result of which the heart rate decreased to 18 in 10 seconds; stimulation of the saphenous now caused the rate to increase to 26½ beats, but after the stimulation it returned to 18.

Repeated stimulation, or sometimes a single stimulation of a sensory nerve, tends to cause permanent (*i. e.* permanent for the individual experiment) changes in the heart rate. Thus, if the heart is beating slowly, two or three stimulations of a sensory nerve may cause an acceleration of the heart which continues for hours, or as long as the experiment continues; simply ligating the sciatic nerve has caused a similar effect. If, on the other hand, the heart is beating rapidly, repeated stimulations may cause the rate to become and remain slow.

These are further illustrations of the already mentioned tendency of the heart to reach and remain at a constant rate, whenever its rate has been changed.

The afferent nerve fibres by which reflex acceleration is produced. —Attention has already been called to the part which the condition of the cardio-inhibitory centre plays in determining whether reflex acceleration or reflex slowing of the heart follows stimulation of afferent nerves: in this section some facts relating to the afferent nerve fibres themselves will be discussed.

Aside from such nerves as the depressors and vagi, which are known to contain nerve fibres having special relations to the vasomotor and respiratory centres, there is evidence that there are different kinds of afferent nerve fibres in other nerve trunks.

In a previous paper, for example, I have collected evidence based on experiments of Howell and others and of my own which tends to show that in most mixed nerve trunks there are two varieties of nerve fibres which may influence the vasomotor centre, one causing a reflex rise, the other a reflex fall of blood pressure.[1] These results suggest the question whether those nerve fibres, stimulation of which causes a reflex acceleration of the heart, will, under different circumstances (when, for example, the condition of the cardio-inhibitory centre has been altered), cause a reflex slowing, or whether, in fact, there are two sets of nerve fibres, stimulation of one of which will always cause an acceleration, provided it has any effect at all upon the heart rate, while stimulation of the other set will cause a slowing. Further, what is the relation of the nerve fibres which cause reflex changes in the heart rate to those which cause changes in the blood pressure?

In the historical review given at the beginning of this part of the paper evidence was stated that stimulation of some nerves (*e. g.* the trigeminus) nearly always causes a reflex slowing of the heart, whereas stimulation of certain other nerves (*e. g.* the sciatic) more frequently causes an acceleration, or an acceleration first and then a slowing. I have observed in my own experiments that when the saphenous and the sciatic were stimulated in the same animal the former usually caused only an acceleration, while the latter frequently caused an acceleration followed by a slowing; moreover, slowing alone occurred more frequently from stimulation of the sciatic than of the saphenous. Since this difference of action of the various nerves was observed in experiments in which there was no reason for supposing that the condition of the cardio-inhibitory centre was different in one case from its condition in the other, the most probable explanation is that there are two varieties of nerve fibres involved, and that one variety occurs in larger number in some nerves than does the other variety.

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[1] HUNT: Journal of physiology, 1895, xviii, p. 381.

The fact also that stimulation of such a nerve as the sciatic has or may have a twofold action upon the heart, causing first a reflex acceleration and then a slowing,[1] suggests that there are two sets of fibres involved, and that the action of one set becomes evident before that of the other.[2] Such a double effect upon the heart is never, or only very rarely, observed when certain other nerves, such as the depressor or trigeminus, are stimulated.

Working upon this supposition, many experiments were performed in the hope of finding a method by which the conductivity or irritability of one set of fibres might be suspended while that of the other remained. The methods employed were in general the same as those which I employed to separate physiologically those nerve fibres which cause a reflex fall of blood pressure from those which cause a rise, and consisted essentially in subjecting the nerve trunks to influences which altered their conductivity or irritability, and then observing the effect upon the heart rate of stimulating them in various ways; of course precautions were taken to avoid causing, at the same time, changes in the cardio-inhibitory centre. Some of the methods employed, although giving evidence of the existence of two sets of fibres to the vasomotor centre, gave only negative results as to the afferent fibres to the cardio-inhibitory centre; one, however, the experiments on the regeneration of nerves, gave positive results.

Effect of stimulating the central end of a recently regenerated nerve. — Most of these experiments were performed upon the sciatic nerves of cats. One nerve was crushed by drawing a ligature tightly around it, high in the thigh; the wound was closed and the nerve allowed to regenerate. After a period of five or six weeks, or after signs of motion became apparent for some distance down the leg, the animals were anæsthetized (usually by cerebral compression), both sciatics exposed and divided, and the central ends stimulated with tetanizing currents of varying intensities. Six experiments were performed in this manner, and in all of them stimulation of the regenerated nerve produced a different effect upon the heart rate from that caused by stimulating the normal nerve. Stimulation of the former usually caused an acceleration of the heart; in some cases no effect was produced, but in none was there a reflex slowing. Stimulation of the normal nerve in these animals caused in all cases a reflex slowing.

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[1] If, as is often the case, a rise of blood pressure also occurs from stimulation of the sciatic, this may be the cause, in part, of the slowing of the heart; the latter often occurs, however, when there is no change in the blood pressure, and also when there is a fall of blood pressure.

[2] Cf. MELTZER: *Archiv fur Physiologie*, 1892, p. 340 (afferent nerves to the respiratory centre); also my paper on afferent nerve fibres to the vasomotor centre, *op. cit.*, p. 406.

One of these experiments was as follows.

Experiment 32. Cat. Left sciatic crushed 42 days previously. Anæsthesia caused by cerebral pressure. Blood pressure 100 mm. of mercury. Stimulation of the left sciatic about 1½ inches below point of injury, with currents of varying strength, caused a very slight acceleration of the heart; in one case, for example, the rate increased from 27 to 29 in 10 seconds, the blood pressure remaining unchanged. Stimulation of the right (normal) nerve caused only reflex slowing; during one stimulation, for example, the rate decreased from 27½ to 20 in 10 seconds, while the blood pressure fell 2 mm. of mercury.

The results of the above experiment differ from those usually observed in that stimulation of the nerves in this case caused no change in the blood pressure, whereas stimulation of the normal nerve usually causes a rise, and stimulation of the regenerated nerve a fall, of blood pressure. The absence of any changes in the blood pressure in this experiment makes it improbable that blood pressure changes were the cause of the difference in the effect upon the heart rate in the other experiments; there is, moreover, other evidence for this view. Thus, if an unusually long interval be allowed to elapse between the crushing and the stimulation of the nerve, the power of a portion of the nerve near the point of injury to cause a reflex fall of blood pressure may be lost, while that to cause acceleration remains. Thus, in one experiment 48 days were allowed to elapse between the operation and the stimulation of the nerves; the result was that stimulation of the nerve at a certain distance from the point of injury caused a rise of blood pressure, while the heart rate was increased from 18 to 24 beats in 9 seconds. Also, on the other hand, stimulation of the normal nerve may occasionally cause a fall of blood pressure, although a reflex slowing of the heart occurs.

I think the above considerations show sufficiently clearly that the changes in the heart rate are independent of the changes in the blood pressure.

The most probable explanation of the result of these experiments is that in such mixed nerve trunks as the sciatic, there are two sets of afferent nerve fibres to the cardio-inhibitory centre, one of which causes inhibition, the other stimulation, of this centre, and that in a nerve which has been crushed the former regenerates more rapidly than do the latter. Of course it is not necessary to suppose that the only function of these nerve fibres is to affect the cardio-inhibitory centre; they are probably ordinary sensory nerve fibres which can cause other reflexes, only some of them are connected with the vagus centre in such a way that their stimulation inhibits, while that of the other excites, this centre. What little evidence there is to connect either of these varieties of nerve fibres with other varieties (those causing changes in the vasomotor centre, for example) will be given below.

Effect upon cardiac reflexes of cooling the afferent nerve. — One of the simplest ways of separating physiologically the nerve fibres, stimulation of which causes a reflex rise of blood pressure from those which cause a fall, is to cool the nerve trunk and then stimulate it below the point of cooling; the reflex vaso-constrictors lose their conductivity at a higher temperature than do the reflex vaso-dilators. My experiments so far have failed to show any difference in the action of cold upon the fibres causing a reflex slowing and those causing a reflex acceleration of the heart. Cardiac reflexes, whether acceleration, slowing, or the two combined, as a rule have disappeared at about the temperature (10° C. or less) at which a reflex rise of blood pressure was no longer obtained; when the cooling was continued below this point stimulation of the nerve caused a fall of blood pressure but no effect upon the heart rate.

Effect of varying the strength and rate of stimulation. — My observations upon these points were confined almost exclusively to experiments upon the sciatic of the dog. When stimulation of this nerve causes a reflex acceleration, there is usually a certain strength of current, varying in different cases, which gives the maximum acceleration, that is, causes the greatest number of additional heart beats. If the strength of the current is reduced below this optimum degree, the maximum acceleration reached during stimulation may not at first be decreased, but the acceleration does not continue so long after the stimulation ceases; if, however, the strength of the stimulus is still further reduced, the maximum acceleration becomes less. When, on the other hand, the strength of the current is increased beyond the optimum, there may be at first a slight increase in the maximum acceleration reached, but this is followed by a greater tendency to slowing. This tendency to slowing becomes, as a rule, more and more marked as the strength of the current is increased, and sometimes stimulation produces only slowing; the latter result, however, is rather infrequent. As a rule, if there has been an acceleration with a weak stimulus, there is an equally great or greater acceleration with a stronger stimulus; the tendency to subsequent slowing is greater in the latter case.[1]

As regards the strength of current necessary to cause reflex changes in the heart rate, as compared with the strength necessary to cause other reflex effects, it may be said that, in general, when the vagus is in tonic activity and the vasomotor centre is irritable, a stimulus too weak to affect the one is also usually without effect upon the other. Frequently neither the heart rate nor the blood pressure is influenced by a stimulus which causes respiratory and other reflex movements.

The results of stimulating a sensory nerve with a succession of induction shocks slowly repeated do not differ materially from those obtained with tetanizing currents; the only difference is that with the former method the acceleration or slowing is more slowly developed.

The above experiments and considerations seem to me to afford clear evidence for the view that there are two varieties of afferent nerve fibres to the cardio-inhibitory centre, one causing an excitation and the other an inhibition of this centre. The mere fact that in the same animal stimulation of one nerve may cause a reflex slowing and stimulation of another, a reflex acceleration of the heart, when the condition of the centre has undergone no change, is difficult to explain on any other hypothesis. Moreover, in the regeneration method we have a means of showing the presence of two sets of nerve fibres in the same nerve; when the nerve is crushed and allowed to regenerate, those fibres causing an inhibition of the centre regenerate earlier than do those which cause an excitation. If the cardio-inhibitory centre underwent changes in irritability in the course of the experiment, it would be easy to see how stimulation of the same nerve fibres might in one case cause inhibition and in another excitation, just as the condition of the cerebral cortex or of the cardiac muscle is supposed to determine whether stimulation shall cause inhibition or augmentation; but in the above experiments changes in the centre were excluded.

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[1] Simanowsky is quoted (Jahresbericht der Anatomie und Physiologie, 1881, x, p. 62) as having observed that stimulation of the brachial and sciatic plexuses with a weak stimulus caused an acceleration, while a strong stimulus caused a slowing of the heart. MacWilliam (Proceedings of the royal society London, 1893, liii, p. 471) obtained similar results.

We have, further, no evidence that stimulation of the one set of fibres can ever under any circumstances cause any change but a reflex slowing, or stimulation of the other set anything but a reflex acceleration of the heart.

Although, as was shown above, the condition of the cardio-inhibitory centre plays an important part in determining the manner in which this centre responds to the stimulation of a mixed nerve, such as the sciatic, yet this influence is limited to determining whether the centre responds to the impulses reaching it along the one or the other set of fibres; in other words, when both sets of fibres are stimulated inhibition or increased action will result according as the centre is more irritable to the one or the other set of impulses.

The relation which these fibres to the cardio-inhibitory centre bear to other nerve fibres — those to the vasomotor centre, for example — is an interesting but very complex problem. The experiments on regenerating nerves suggested at first that the fibres which cause a reflex fall of blood pressure are identical with those causing a reflex acceleration of the heart. The experiments on the effect of cold upon the nerves, however, show that the two sets of fibres cannot be identified in this manner, for cold causes the fibres which produce reflex changes in the heart rate to lose their conductivity earlier than those which cause a fall of blood pressure.

For a similar reason it seems impossible to identify the fibres which cause a rise in blood pressure with those which cause a slowing of the heart.

Of course the relative irritability of the cardio-inhibitory and vasomotor centres probably exerts an important influence in this matter, and a more thorough investigation may show the following view to be incorrect, but at present it is difficult to avoid the conclusion that we are dealing with four varieties of nerve fibres: (1) those causing a reflex rise of blood pressure, (2) those causing a reflex fall of blood pressure, (3) those causing a reflex slowing of the heart, and (4) those causing a reflex acceleration of the heart. These four varieties of nerve fibres are very unequally distributed in different nerve trunks, some being entirely absent from certain of them. Thus there is no satisfactory evidence that there are fibres in the depressor which ever cause a reflex rise of blood pressure,[1] or that the saphenous of the cat contains any fibres which can cause a fall;[2] as has already been pointed out, stimulation of the trigeminus, if it has any effect upon the heart, always causes a slowing, and v. Cyon[3] found only an acceleration to result from stimulation of the third root of the depressor. The glossopharyngeal usually causes a fall of blood pressure and a slowing of the heart, the infraorbital[4] a rise of blood pressure and a slowing of the heart, while stimulation of the sciatic may cause either a rise or a fall of blood pressure and either a slowing or an acceleration of the heart.

PART III.

GENERAL CONSIDERATION OF RESULTS.

Functions of the accelerator nerves. — In speaking of the functions of the accelerator nerves it is necessary to distinguish the effect which these nerves have upon the rate from their effect upon the force of the heart-beat. It is well known that these two effects have no constant relation to each other; in fact they seem almost always to occur separately. Thus in very few of my own experiments in which acceleration of the rate has been observed has there been any evidence that the force of the beats has been increased; a rise of blood pressure was exceptional, and when present bore absolutely no relation to the increase in the rate. Although the same observation has been recorded by almost every one who has worked upon this subject, the statement is still frequently made that the increase in the heart rate leads to a rise of blood pressure.

On the other hand, in those experiments in which increase in the force of the heart-beat has been found to follow stimulation of the accelerators there has been as a rule little or no acceleration of the rate. Thus in my own experiments when a rise of blood pressure occurred it usually resulted from stimulation of the accelerators of the left side, and as I have already shown these nerves have, as a rule, but little effect upon the heart rate. It is interesting that in the experiments which Roy and Adami,[1] who studied the effect of the accelerators upon the force of the heart-beat very carefully, quote to show the effect of direct stimulation of these nerves upon the heart, there is a marked increase in the force of the heart while there is scarcely any change in the rate.

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[1] See v. CYON: Archiv f. d. ges. Physiol., 1898, lxx, p. 229.

[2] HUNT: Journal of physiology, 1895, xviii, p. 386.

[3] v. CYON: *op. cit.*, p. 142.

[4] KNOLL: Sitz.-Ber. d. kais. Akad. d. Wiss., math.-naturw. Cl., 1885, xcii, 3, p. 449.

Such facts as the above have led to the suggestion that there are really two kinds of nerve fibres in the accelerator nerves: one which causes increase in the rate, and another which causes an increase in the force of the beat. The former variety may be called the accelerator, the latter the augmentor nerve fibres.

The function of the augmentor fibres is doubtless to cause a rise of general blood pressure, or, as Roy and Adami[2] put it, "they sacrifice the heart in order to increase the output of the organ and enable the ventricles to pump out their contents against heightened arterial pressure."

The principal functions which can be ascribed to the nerve fibres which cause an increase in the rate of the heart with no effect upon the blood pressure have been referred to already when their tonic activity and relation to the vagi were discussed. From the experiments on the effects of cutting these nerves two conclusions may be drawn: (1) the normal rate of the heart is determined in part by the impulses constantly reaching it through these nerves, (2) in cases in which the irritability of the heart is low, the tonic activity of these nerves plays an important part in maintaining the regular rhythm of the heart. Attention was also called to the fact that the accelerator centres and nerves are very resistant to influences (low blood pressure, extreme asphyxia, certain drugs, etc.) which quickly depress other nerve centres and even affect the cardiac muscle itself; hence these nerves, in virtue of their tonic activity, are in a position to supply an efficient stimulus to the heart when one is most needed.

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[1] ROY and ADAMI: Philosophical transactions, 1892, 183 B, p. 244, see curve 14, p. 239, stimulation of left annulus, and curve 15, p. 241. In most of the experiments upon which these authors base their views of the effect of the accelerators upon the force and output of the heart acceleration was produced reflexly by the stimulation of a sensory nerve; as has been shown above it is very improbable that the acceleration in such cases was due to the accelerator nerves at all.

[2] ROY and ADAMI: *op. cit.*, p. 296.

It was shown further that the tonic activity of these nerves limits the action of the vagi; when the heart is slowed by reflex stimulation of the vagus, not only do the accelerators limit the extent of the slowing, but they enable the heart to return more quickly to its normal rate. It is probable that this action of the accelerators is very important in counteracting influences which cause reflex slowing of the heart, such, for example, as injury to the abdominal viscera, and perhaps also in asphyxia.

Nothing definite can be said as to the part the accelerators play when they are thrown into increased activity, as so little is known about the conditions under which this occurs; in fact, little can be added to the statements made by the brothers Cyon more than thirty years ago. These physiologists, who recognized that there is not necessarily an increase in the work done when these nerves are stimulated, suggested that their function consisted in diminishing the resistance which the vagi opposed to the development of the heart-beat.

Although the total amount of work done by the heart when it is accelerated by stimulation of these nerves is not increased (the work being simply differently distributed in time, as the Cyons expressed it), yet, as has been already shown, this acceleration causes fatigue of the heart; perhaps this fatigue is due to the shortening of the periods during which the heart is at rest. In what manner either the heart itself, or the rest of the body, benefits by the more rapid rate (which of itself causes fatigue of the heart) is obscure.

Possibly some clue to this problem is to be found in the work on the transfusion of blood through isolated organs. It has become the generally recognized view that in such experiments much better results are obtained when the stream of blood is supplied intermittently[1] than when it is supplied at a constant pressure. If the blood is supplied under constant pressure, œdema and other pathological changes appear in the organ under experiment,[2] and the circulation is soon slowed, or even arrested altogether; this is due in part to the corpuscles adhering to the walls of the blood vessels and to each other,[1] and even a great increase in the pressure causes but a temporary improvement. If, however, the blood is supplied intermittently, the slight movement of the corpuscles at each pulsation and the consequent changes in the diameter of the blood vessels prevent this clumping together of the corpuscles, and the transfusion can be continued for a much longer time at a comparatively low pressure.[2]

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[1] Although Kronecker, in 1871, stated that less injury is done to the vessels of a muscle by high pressure if this is intermittent than when it is constant, Stevens and Lee (Studies from the biological laboratory, Johns Hopkins University, 1884, iii, p. 109) seem to have been the first actually to make use of an intermittent supply of nutrient fluid in transfusion experiments.

[2] HAMEL: Zeitschrift für Biologie, 1889, xxv, p. 492.

I have been unable to find any statements as to the number of pulsations per minute which yield the best results in transfusion experiments; this number would probably be influenced by such factors as the nature of the blood-vessel walls, the length of the capillaries, etc., so that it is very probable that a rate which is suitable for one organ would not be adapted to another. Support for this supposition is found in v. Cyon's work on the circulation through the thyroid gland: [3] v. Cyon showed that when the heart was beating slowly, as a result of stimulating the vagus, a much greater amount of blood flowed from the thyroid vein than when the heart was beating more rapidly, although the blood pressure was the same and vasomotor changes in the gland were excluded; the outflow from the saphenous vein was also increased, but relatively to a much less degree than that from the thyroid vein.

In the light of such experiments as the above it seems quite probable that conditions may arise under which the circulation of an organ may be better provided for by a series of rapid heart-beats, each of which throws out a smaller quantity of blood, than by a slower rate with which the output at each beat is greater. In fact certain changes in the respiratory waves of the curve of blood pressure resulting from the stimulation of the accelerator nerves seems to point to such an action. One of the most constant effects upon the blood pressure curve of stimulating the accelerator nerves is a marked increase in the amplitude of the respiratory undulations; this occurs not only when the blood pressure rises or remains at the same level, but also when it falls; and further, it occurs in animals under curare and in which artificial respiration is maintained by a pump or pair of bellows which discharges with great regularity the same amount of air at each stroke. If changes in both the blood pressure and the volume of the respired air are excluded, as is the case in such experiments as these, then the increased amplitude of the respiratory waves seems to indicate that a larger volume of blood is passing through the lungs at any given time, and this, in turn, that a larger amount of blood is being returned to the right auricle through the systemic vessels. Of course the increased amplitude of these waves may have been due in part to the faults of the mercury manometer, that is, when the heart was beating more slowly the excursions of the column of mercury were increased so greatly by its inertia that the respiratory undulations were obscured, whereas with the more rapid rate the effects of the mercury's inertia were less marked. But it does not seem probable that this change in the respiratory waves can be explained entirely in this manner, for not only are these waves often marked when the heart is beating slowly, but their amplitude is frequently increased by injecting a large amount of normal saline solution into the circulation, although no changes occur in the blood pressure and heart rate, or at least do not occur for some time after the changes in the respiratory waves. The increased amplitude of these waves after the injection of normal saline solution is almost certainly due to the larger amount of liquid in the vessels of the lungs; the same change occurring after stimulation of the accelerators is probably due to a similar cause.

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[1] von Frey: Archiv für Physiologie, 1885, p. 538. Welch and Mall consider that the absence of pulsation in an occluded artery plays an important part in the production of a hæmorrhagic infarction.

[2] Jacobi: Archiv f. exper. Pathol. u. Pharmakol., 1890, xxvi, p. 398.

[3] v. Cyon: *op. cit.*, p. 161.

Finally the experiments of Stevens and Lee upon the action of intermittent pressure upon the blood vessels of the frog and terrapin suggest the possibility that the accelerators may, at times, play a part in maintaining the normal tone of the blood vessels. These authors found that " a rhythmically interrupted force applied to the blood vessels of the frog and terrapin through the medium of a circulating fluid exerts a special action upon them, in consequence of which a constriction of them takes place; " as a result of such action a much smaller amount of liquid supplied intermittently is needed to maintain a given arterial pressure than when the force is a constant one. No experiments were made to determine the number of interruptions which give the best results; it is probable, however, that this number would be found to differ in the case of the vessels of different organs, so that in some a rapid, in others a slow, rate would have the more marked effect.

Rapid heart action of other than reflex origin. — There are many cases of rapid heart action of other than reflex origin upon which the experiments described in this paper may throw some light; a few such cases will be considered briefly.

Voluntary acceleration of the heart. — The power which some persons have of voluntarily increasing the heart rate has been studied with especial care by Tarchanoff[1] and Pease,[2] and more recently by Van de Velde.[3]

Tarchanoff made a number of experiments in order to determine the cause of the acceleration, *i. e.* whether it is due to a diminution of the tonic activity of the vagi or to a stimulation of the accelerators; he reached the conclusion that the acceleration is due to a direct action upon the centres of the accelerator nerves. The grounds for this conclusion were three: (1) the manner in which the acceleration appeared and disappeared, (2) the changes in the form of the sphygmograms, and (3) the changes in the volume of the extremities. Tarchanoff found that in the person upon whom he experimented, the maximum acceleration was reached only after one half to three quarters of a minute after the beginning of the effort to increase the heart rate; the return to the normal rate was also gradual. Comparing the slow development of the voluntary acceleration with the rapid development following the section of the vagi, Tarchanoff drew the conclusion that the voluntary acceleration was due to the accelerator nerves. It does not seem to me that this argument is at all conclusive. It does not seem fair to compare the effect of section of the vagi, which causes an immediate effect on the heart rate, with the acceleration following a distinct effort of the will.[4]

It has also been shown above that under certain circumstances the diminution of the tonicity of the vagus caused by the stimulation of a sensory nerve may be very slowly developed and very slowly disappear. Moreover, in one of Van de Velde's patients the maximum acceleration occurred in the first 10 seconds of the effort; whereas Böhm in experiments upon animals found from the examination of a large number of tracings that when the accelerators were stimulated directly the maximum acceleration occurred as a rule in the second

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[1] TARCHANOFF: Archiv f. d. ges. Physiol., 1885, xxxv, p. 109.

[2] PEASE: Boston medical and surgical journal, 1889, cxx, p. 526.

[3] VAN DE VELDE: Archiv f. d. ges., Physiol., 1897, lxvi, p. 232.

[4] Van de Velde compares the effort necessary to cause acceleration to the feeling experienced when a person attempts to perform some unusual muscular movement, such, for example, as contracting the muscles of the ear, or flexing independently the last phalanx of the finger.

10 seconds of the stimulation. In the experiments of Pease also the latent period seems to have been very short; thus in some of the tracings given the first beat after the effort was made seems to have been shortened and the greatest acceleration to have occurred in the first two or three seconds, the entire effort continuing but five seconds. We certainly have no reason to suppose that the accelerators can be thrown into activity by an act of the will more quickly than when they are stimulated directly by the electric current.

It is also worthy of note that in the case of Salomé, upon whom most of Tarchanoff's experiments were made, acceleration of the heart easily resulted from slight external causes, — a fact which, judging from experiments upon animals, points to an unstable condition of the cardio-inhibitory centre.

The second argument which Tarchanoff brings forward in support of his view — the changes in the form of the sphygmogram — seems to me to be still less conclusive, owing to the difficulty of interpreting these curves; in fact the changes which Tarchanoff adduces as evidence that the increase in the heart rate was due to the action of the accelerators are very nearly the same as those which Nothnagel brought forward to show that in some cases of rapid heart action the increase comes from a diminution of the vagus tonus.

The third argument of Tarchanoff in support of the above view is that when the heart rate increased, the volume of the foot, as determined by the plethysmograph, was not increased, although, according to Tarchanoff, an increased volume would necessarily have occurred as a result of the augmented blood pressure if the acceleration in rate had been due to a diminution of vagus tonus; it is however, by no means uncommon for section of the vagi to cause acceleration of the heart without any increase in the blood pressure occurring — in fact there is sometimes a fall of general blood pressure.

In the light of these considerations it seems to me that the evidence for the view that voluntary acceleration is due to the action of the accelerator nerves is entirely inconclusive; in fact I am inclined to think that the weight of evidence is rather in favor of the view that it is due to a diminution of the tonus of the vagi.

Acceleration of the heart during muscular exercise. — The influence of the cardiac nerves on the increase in the heart rate during muscular exercise has been most carefully studied by Hering.[1] This author reaches the conclusion that the acceleration is due in part to an increased activity of the accelerator nerves, perhaps of reflex origin, and in part to a diminution of the tonic activity of the vagi caused by the increased respiratory movements.

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[1] Hering, H. E.: Archiv f. d. ges., Physiol., 1895, lx, p. 429.

It seems to me that a careful examination of Hering's experiments shows that the diminution of the tonic activity of the vagi is sufficient to explain his results, and that there is no clear evidence for believing in any increased action of the accelerators; the tonic activity of the latter nerves plays, however, a very important part here, as in the acceleration following stimulation of sensory nerves. Without going into too many details, the following observation on Hering's experiments may be made. The average rate of the heart reached during muscular exercise in a number of experiments was 320 beats per minute, whereas the average rate during rest after section of the vagi was 321. Moreover, in one half of these experiments (see table p. 440) the maximum rate reached during exercise was less than or just the same as the rate in rest after the vagi were cut; in the other half of these experiments the maximum acceleration in exercise after the vagi were cut was less than 7 per cent. This acceleration of 7 per cent may have been due to a stimulation of the accelerator nerves, but it should be observed (1) that in other experiments (p. 476) in which both the vagi and accelerators had been cut a much greater acceleration (33 per cent and more) — due probably to changes in the respiration and blood pressure — was observed, and (2) that direct stimulation of the accelerators after section of the vagi may cause a much greater increase in the heart rate; the Cyons, for example, obtained an acceleration of over 50 per cent in such experiments.

On the other hand Hering's results show very clearly that the acceleration of the heart rate in exercise was much less after section of the accelerators; the average acceleration was but two-fifths as great as when they were intact. I think, however, that this difference can be attributed entirely to the removal of the tonic impulses of the accelerators. That the accelerators were in tonic activity in these experiments is made almost certain by the fact that when the vagi were cut after section of the accelerators there was but a slight increase in the heart rate; the greatest increase was 60 and the average 31 beats per minute, whereas in another series of experiments in which the vagi alone were cut the average increase was 122 beats and the smallest increase (and this was a very exceptional case) was 68.[1] It seems to me that these results, so far as the influence of the cardiac nerves is concerned, can be explained in exactly the same way as the acceleration following stimulation of a sensory nerve, namely, the essential factor in the acceleration is a diminution of the activity of the cardio-inhibitory centre; if the accelerator nerves are intact and in tonic activity the maximum rate reached is greater than when they are not in activity, but there seems to be no sufficient evidence for supposing that these nerves are thrown into increased action in muscular exercise.

Athanasiu and Carvallo[2] have also recently studied this question, and reached the conclusion that the essential factor in the acceleration is the reflex diminution of the activity of the cardio-inhibitory centre; their tracings also show that the latent period of the acceleration in muscular exercise is very short.

MacWilliam,[3] who believes the acceleration to be of vagus origin, has called attention to the fact that in those animals which are capable of long continued muscular exercise (such as the horse, dog, and hare) the vagi are in a condition of marked tonic activity, while in such an animal as the rabbit, which is not capable of so prolonged exertions, the tonic activity of the vagi is not so marked.

Acceleration following compression of the carotids. — Cooper and Magendie stated that acceleration of the heart results from the compression of the carotid; this subject has been studied with especial care by François-Franck,[4] who considered acceleration to be due to a stimulation of the accelerator nerves. Examination of the curves and the descriptions of the experiments of François-Franck show that in most cases the vagi were intact; in other cases nothing is said about the vagi. The curves published by this author are strikingly like the curves of reflex acceleration obtained by stimulating a sensory nerve after section of the accelerators; the second, and in some cases the first, heart-beat after compression of the carotid was shortened, and after the compression ceased the heart returned instantly to its previous rate or was slowed, the very first beat being either of the normal length or prolonged. François-Franck noted the very short latent period as compared with the latent period when the accelerator nerves are stimulated electrically, and suggested that the difference may be due to the normal stimulus from the central nervous system acting differently from direct electrical stimulation; but there seems to be no evidence for this supposition. It is true that François-Franck states that no acceleration occurred after extirpation of the inferior cervical and first thoracic ganglia; but such an operation, unless performed with great care, destroys the tonicity of the vagus centre.

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[1] Hering commented on the slight increase in the heart rate following section of the vagi after previous section of the accelerators, and recognized that the integrity of these nerves is an important factor in the acceleration following section of the vagi, but he seemed to hesitate to interpret his results as evidence for the tonic activity of the accelerators. (See also note on p. 397.)

[2] ATHANASIU and CARVALLO: Archives de physiologie, 1898, p. 561.

[3] MACWILLIAM: Proceedings of the royal society, London, 1893, liii, p. 476.

[4] FRANÇOIS-FRANCK: Travaux du laboratoire de Marey, 1878–79, p. 74.

Many more cases of acceleration of the heart in which diminution of the tone of the vagi is probably the chief factor might be cited. Thus the acceleration accompanying each act of deglutition in man,[1] that occurring during each inspiration,[2] and the increase in the heart rate when the lungs are inflated by a pair of bellows,[3] all seem to be due to a diminution of the tonic activity of the vagi. The cause of the rapid heart rate observed in certain diseases,[4] and after the admin-

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[1] MELTZER: Archiv für Physiologie, 1883, p. 223.

[2] FREDERICQ: Archives de biologie, 1882, iii, p. 86.

[3] HERING: Sitz.-Ber. d. kais. Akad. d. Wiss., math.-naturw. Cl., 1871, lxvi, 2, p. 348. See also H. E. HERING: Archiv f. d. ges. Physiol., 1895, lx, p. 461.

[4] The rapid heart action in paroxysmal tachycardia is sometimes referred to a diminution of the tonus of the vagus, sometimes to a stimulation of the accelerators; there seems to be little unanimity of opinion among those who have described this condition. The above experiments upon animals seem adapted to throw some light upon this subject. Unless we assume that the accelerator nerves of man are very different physiologically from those of the lower animals, it seems impossible to accept the view that in some cases of tachycardia the rapid rate of the heart is due to a stimulation of the accelerator nerves. Thus there are cases reported (by H. C. Wood, *e. g.* University medical magazine, 1890–91, iii, p. 273, and by Hochhaus, Deutsches Archiv für klinische Medicin, 1893, li, p. 19) in which the rapid action of the heart ended instantaneously, the rate decreasing in one case from 184 to 78 per minute; experiments on animals show that stimulation of the accelerators is always followed by a long after-effect upon the heart. Again, the rapid action of the heart in these cases may continue for days, the heart beating continuously at double or more than double its normal rate; such an acceleration in animals can be maintained for but a very short time by powerful electrical stimulation of the accelerators.

On the other hand, many of these cases can be easily explained by the diminution of the tonus of the inhibitory nerves. I have shown above how easy it is to get the cardio-inhibitory centre into a condition of unstable equilibrium by the action of drugs, changes in blood pressure, respiration, etc.; I have also called attention to the fact that there are great individual variations, the centre being inhibited in some animals with ease, in others of the same species with difficulty. When the cardio-inhibitory centre is in such a condition of unstable equilibrium, very slight istration of drugs, is in many cases obscure; but there seems as yet to be no evidence inconsistent with the view that in these cases the action is, as a rule, either a direct one upon the heart itself or upon the cardio-inhibitory centre.

Unusual difficulties are met with in investigating the effect of psychical influences upon the heart rate, but there seems to be no reason for supposing that the acceleration in such cases is different in origin from that in the cases which have been discussed above.

Leaving out of consideration the cases in which the cause of the acceleration is doubtful, and admitting that in some of those discussed above the acceleration is due in part to a direct action of the accelerator nerves, it must still be granted that decrease of the tone of the cardio-inhibitory centre is the most usual cause of an increase in the heart rate.

When it is remembered what an important part inhibition in one form or another plays in other functions of the body, — how, for example, it seems to occur in every act of respiration,[1] in every movement of a limb,[2] how important it is in movements of the pupil,[3] the regulation of the flow of the blood, the movements of the intestine,

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causes — a little ether or curare, weak stimulation of a nerve, a little operating, etc. — are sufficient to cause a marked acceleration of the heart; this acceleration may continue for a long time. If we assume that in the patients who suffer from paroxysmal tachycardia the cardio-inhibitory centre is in an unstable condition (usually as an individual peculiarity, though this condition seems to be exaggerated at times by such influences as recent illness, excessive use of alcohol, coffee, etc.), it is easy to see how some slight change might bring on an attack. Among the exciting causes mentioned (see Pröbsting, Deutsches Archiv für klinische Medicin, 1882, xxxi, p. 349, and Herringham, Edinburgh medical journal, 1897, i, p. 367) are sudden exertions or injuries, digestive and uterine disturbances — influences which might readily cause a reflex inhibition of the vagus centre. In some cases pathological changes of the pericardium and of the myocardium are described; in the light of the experiments of Knoll upon the reflexes from the heart it is not improbable that such changes can cause a reflex inhibition of the cardio-inhibitory centre. That diminution of the tonic influence of the vagi is sufficient to account for the rapid heart observed in most cases of tachycardia follows from the effects of injuries to the trunk of the vagi; Edinger says that a heart rate of 240 per minute and more has resulted in man from such injuries — a rate which is not often exceeded in paroxysmal tachycardia.

[1] MELTZER: Archiv für Physiologie, 1892, p. 340.

[2] HERING and SHERRINGTON: Archiv f. d. ges. Physiol., 1897, lxviii, p. 222. SHERRINGTON: Journal of physiology, 1899, xxiii, suppl., p. 26.

[3] BRAUNSTEIN: Zur Lehre von der Innervation der Pupillenbewegung, 1894, p. 95.

etc.,[1] it is not surprising that the body should make use of it in regulating the rate of the heart-beat. Not only can an increase in the heart rate be caused more quickly by an inhibition of the cardio-inhibitory centre than by a stimulation of the accelerator centre, but it probably involves a smaller expenditure of energy. Although the accelerator nerves take no direct part in the production of a more rapid heart-beat (*i. e.* they are not thrown into increased activity), yet it is largely in virtue of their tonic activity that inhibition of the vagus centre leads to such prompt results.

Whether an inhibition of the accelerators leading to a slowing of the heart ever occurs is not known. It is not improbable, however, that changes in the tonicity of the accelerator centre occur, the tonicity being at one time increased and at another decreased, but there seems to be no evidence that such changes are ever produced suddenly, for example, as a result of a reflex act.

SUMMARY OF RESULTS.

The chief facts brought out in this paper may be summarized as follows: —

(1) The accelerator nerves of the dog, cat, and rabbit, and probably of other mammals, are almost always in tonic activity; the tonic activity of the accelerators is not impaired by influences which abolish not only the tonic activity but also the irritability of many other nerve centres.

(2) Under some circumstances the tonic activity of the accelerators is an important factor in maintaining the normal rhythm of the heart, and stimulation of them will sometimes cause an irregular heart to become regular; this action of the accelerator seems to be most marked in cases in which the irritability of the cardiac muscle has been reduced in some manner.

(3) Stimulation of the accelerators, if excessive, will decrease the rate of beat, and make the heart more irritable to impulses reaching it through the vagi. Even death may result from the excessive stimulation of these nerves.

These facts indicate that the stimulation of the accelerators can cause real fatigue of the cardiac muscle.

(4) New evidence is afforded for the view that the inhibitory and accelerator nerves are strictly antagonistic; this antagonism applies to

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[1] For other illustrations of inhibition, see MELTZER: Archiv für Physiologie, 1883, pp. 225–235.

both systole and diastole, but with the same strength of current the systole is more easily influenced by stimulation of the accelerators, and the diastole by stimulation of the inhibitory nerves.

(5) The tonic activity of the accelerators limits the tonic activity of the inhibitory nerves, and *vice versa ;* the normal heart rate, so far as this is governed by the extra-cardiac nerves, is determined by the impulses reaching it simultaneously through these two channels.

(6) The inhibitory nerves exert a protective influence over the heart; not only do they restrain the action of the accelerators, but their stimulation seems to improve the condition of the heart.

(7) The view that reflex acceleration is caused by inhibition of the cardio-inhibitory centre is confirmed; no evidence could be found that the accelerator nerves are ever thrown into action reflexly.

(8) The tonic activity of the accelerator nerves exerts a modifying influence over reflex acceleration; the maximum acceleration is greater, and is longer continued, and there is less tendency to a subsequent slowing when these nerves are intact.

(9) There are probably in most nerve trunks two varieties of nerve fibres to the cardio-inhibitory centre; one variety causes an inhibition, the other an excitation of this centre. When a nerve is crushed the former variety regenerates earlier than the latter.

(10) The most important functions of the accelerators seem to be connected with their tonic activity; aside from this their functions are obscure, but it is not improbable that by altering the rate of the heart-beat they exercise an important influence upon the circulation through certain organs.

(11) It is very probable that almost all cases of rapid heart action are due to a diminution of the tonic activity of the cardio-inhibitory centre.

THE PHYSIOLOGICAL ACTION OF EXTRACTS OF THE SYMPATHETIC GANGLIA

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THE presence of peculiar polygonal cells which stain deeply in chromic acid or its salts in the medulla of the suprarenal bodies and in the sympathetic ganglia of the higher vertebrates has been recently pointed out by Stilling,[1] Kose,[2] and Kohn.[3]

Many embryologists are of opinion that the medulla of the adrenals is developed in connection with the sympathetic nervous system and from the same Anlage. The first to notice this connection were Bergmann[4] and Remak.[5] Leydig[6] showed the intimate connections between the sympathetic and the paired suprarenal glands of Elasmobranchs. Balfour[7] confirmed this and described in Elasmobranch fishes a series of paired bodies derived from the sympathetic Anlage and an unpaired body of mesoblastic origin — the mesoblastic portion represents the cortical part of the matured suprarenal body, while the paired bodies from the sympathetic Anlage form the medulla. Brun[8] has established the development of the medullary cells from the sympathetic nerve cells in Reptilia also. Mitsukuri[9] confirms the above observations of Balfour on the separate origin of the two portions of the adrenals in Elasmobranchs and shows further that in mammals the medullary portion insinuates itself gradually into the cortical organ, although at first separate from and outside it, but continues to retain its intimate connection with the neighboring sympathetic ganglia. Recently Swale Vincent[1] has described in the medullary organs of Elasmobranchs a peculiar cell which he believes to be a transition form between a nerve cell and a medullary cell. This form is found also in amphibians, reptiles, and birds. The same observer[2] noted further the presence of cells apparently identical with those of the suprarenal medulla in the sympathetic ganglia of Salamandra maculosa. Other embryologists[3] point out that in man and certain other mammals, if not in all, both the cortex and medulla of the adult gland are formed from mesenchymal cells.

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[1] STILLING: Anatomischer Anzeiger, 1898, xv, p. 229.

[2] KOSE: Sitz.-Ber. des deutschen naturw.-medicin. Ver. f. Böhmen, "Lotos" 1898, Nr. 6. I have found no difficulty in recognizing these cells in preparations made by Kose's method.

[3] KOHN: Präger medicinische Wochenschrift, 1898, xxiii, Nr. 17.

[4] BERGMANN: Dissertatio de glandulis suprarenalibus, Göttingen, 1839.

[5] REMAK: Untersuchungen über die Entwickelung der Wirbeltiere, Berlin, 1855.

[6] LEYDIG: Beiträge zur mikroskopischen Anatomie, etc., der Rochen und Haie, Leipzig, 1852.

[7] BALFOUR: A monograph on the development of Elasmobranch Fishes, London, 1878; also Text-book of comparative embryology, 1881, ii, p. 459.

[8] BRUN: Arbeiten aus der zoologischen-zootomischen Institut, Würzburg, 1879, v, pp. 1–30, Table iii.

[9] MITSUKURI: Quarterly journal of microscopical science, xxii, pp. 17–29.

The very general belief in the similarity in structure and origin of the sympathetic cells and the cells of the medulla of the adrenals suggested to me that an extract prepared from sympathetic ganglia, intravenously injected, might have the same powerful action on the blood pressure that Oliver and Schäfer[4] have taught us to expect from extracts of the medulla of the suprarenal bodies. I therefore have made many intravenous injections of the extract of sympathetic ganglia and have found indeed that a very decided change in the blood pressure always takes place, but in the opposite direction to that expected; for suprarenal extract causes the pressure to rise, but an extract of sympathetic ganglia causes invariably a marked fall.[5]

Starting from this point the action of the extract on the peripheral and central vasomotor mechanisms, skeletal and cardiac muscle, and on the isolated heart, has been studied, with the results set forth in the following pages.

The making of the extracts. — The extracts employed in this research were prepared from fresh sympathetic ganglia of the ox, sheep, pig, dog, and cat.[1] In the first three animals only ganglionic masses from the solar plexus were used. In the dog and cat separate extracts were made from several sympathetic ganglia, namely, superior cervical, inferior cervical, stellate, and semilunar ganglia. Each of these several extracts, when injected into the veins, possesses the power of lowering the blood pressure.

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[1] VINCENT: Internationale Monatschrift für Anatomie und Physiologie, xv, p. 288.

[2] VINCENT: *loc. cit.*, xv, p. 298.

[3] See MINOT: Human embryology, 1892, p. 488.

[4] OLIVER and SCHÄFER: Journal of physiology, 1895, xviii, p. 230.

[5] The embryological studies mentioned above suggest that the substance which causes such a marked fall in the blood pressure may yet be found in the medulla of the suprarenals. Its presence there in minute quantities might well be masked by the epinephrin isolated by Dr. Abel (The Johns Hopkins hospital bulletin, No. 76, July, 1897), a body which causes a great increase in blood pressure. It should be remembered that Dr. Abel, while separating epinephrin, did indeed find in the medulla a substance lowering the blood pressure.

Three methods were used to prepare the extracts: (1) the ganglia were cleaned of adherent fat, etc., cut into small pieces, placed in a glass vessel, and covered with chemically pure glycerine. The vessel was now put into a thermostat and the contents allowed to macerate for several days at a temperature of 37° C. When about to be used a small quantity of 0.8 per cent sodium chloride solution was added, and after trituration in a mortar the whole was filtered under pressure. (2) The ganglia were finely divided, triturated in a mortar with a small quantity of clean white sand and a few cubic centimetres of the saline solution, warmed in a test tube, and then filtered. This warming of the extract was several times carried past the boiling point without affecting the activity of the extract. (3) The ganglia were placed in a desiccator over concentrated sulphuric acid and, after being thoroughly dried *in vacuo*, were powdered, mixed with saline solution, and filtered. Of these three methods the first was the one most commonly employed, as it furnished a stock which could be kept for any length of time and used as desired by the simple addition of the saline solution and filtration. In the preparation of these extracts the glycerine or the saline solution was added in the proportion of 1 c.c. to 1 gram of ganglia.

The extracts were administered by direct injection into either the external jugular or the femoral vein. The fall in blood pressure took place immediately. Control experiments were done with the glycerine and saline solutions employed in the research, and with extracts prepared from brain substance, spinal cord, spinal ganglia, nerve (sciatic and vagus), and abdominal tissue — peritoneum, fat, etc. From none of these was a fall in blood pressure obtained.

In all experiments in which a tracing of the blood pressure was taken the animals were anæsthetized with a small dose of morphia and ether. The cannula leading to the manometer was inserted in one of the common carotid arteries — usually the left. The manometer used was the common U-shaped mercury form. The tubes between the manometer and the carotid were filled with a 30 per cent solution of magnesium sulphate.

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[1] One experiment was done with an extract prepared from the semilunar ganglion of a Brazilian monkey, with results similar to those produced by the other extracts.

ACTION ON THE CIRCULATION.

General blood pressure. — In more than sixty injections, made on sixteen cats and five dogs, the extract of sympathetic ganglia invariably lowered the general blood pressure from 10 to 50 mm. Hg according to the dose administered. In the cat 1 c.c. would usually cause a fall of 20 to 30 mm. Hg. The pressure change is almost immediate; indeed when the injection is made very slowly the fall begins before the injection is finished. The characteristic result is shown in Figure 1. The pressure falls rapidly, and before long returns slowly to the original height. The experiment may be repeated a number of times on the same animal. In only one instance did the extract appear to have a prolonged effect. Here the return to the normal was extremely slow. In two animals a slight rise preceded the fall in pressure.

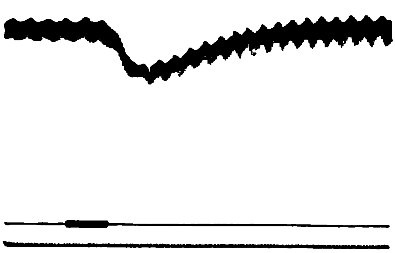

FIGURE 1. One third the original size. The uppermost curve shows the fall in the blood pressure in the carotid artery of the cat, on the injection of sympathetic extract into the jugular vein. The middle line is atmospheric pressure. The heavy black band marks the duration of the injection. The lowest line gives the time in seconds.

In several experiments an endeavor was made to neutralize the depressant action of the extract by injecting with it an extract prepared from the medulla of the suprarenal capsules. The results varied according to the proportion of the extracts; when suprarenal was in excess a rise took place, when sympathetic a fall. In two cases a balance was nearly struck and an interesting tracing was the result, the blood pressure rising quickly for an instant and then as quickly falling again to slightly below the normal, where it remained for a few seconds. This was repeated several times until at last the oscillations ceased and the rise caused by the suprarenal extract remained comparatively steady. In two experiments on cats the cannula of one syringe was tied in the external jugular vein and that of another fastened in the femoral. The former was filled with an extract prepared from the sympathetic and the latter with suprarenal extract. It was found that the fall produced by injecting sympathetic extract could be checked or counteracted by the immediate injection of suprarenal extract. This experiment was reversed by injecting suprarenal extract and checking the consequent rise with sympathetic extract.

The rabbit's ear. — Injections were made into the jugular vein of two white rabbits and the animals' ears held between the observer and the light. A dilatation of the vessels of the ear could be plainly seen; small vessels which were invisible before stood out clearly.

The extraordinary fall in blood pressure which we have seen to follow the injection of the extract of sympathetic ganglia may be the consequence of the action of the extract directly on the vasomotor centres, the sympathetic vasomotor neurons, or the blood vessels themselves, or finally, on any two or perhaps on all three of these structures. Experiments to answer these questions, in part at least, were now made. The first question to be determined is whether the extract dilates the blood vessels through the agency of the vasomotor centre in the bulb.

Perfusion of the bulb. — A cat was anæsthetized with ether and tracheotomized. The subclavian arteries and the two vertebral arteries were tied close to their origin and a ligature was passed round the superior vena cava. In the cardiac end of the left carotid was inserted a cannula connected with a mercury manometer recording the systemic blood pressure on a smoked drum. In the cranial end of the same artery was placed a cannula connected with the perfusion apparatus, arranged to supply at will normal defibrinated blood mixture, or blood mixture containing sympathetic extract. The right carotid artery was laid bare but was left free to supply the bulb with normal blood until perfusion actually began. Both vagi were divided. The ligature about the superior vena cava was drawn tight. The jugular veins were now opened freely, the right carotid artery clamped, and the brain perfused with defibrinated blood at 100 mm. Hg pressure. The blood reached the brain through the cannula in the left carotid artery — the only cerebral artery remaining open — and escaped through the severed jugular veins. None of the brain blood could reach the heart, because the superior vena cava was tied. Thus the blood-supply of the brain was separated entirely from that of the rest of the animal, which continued to be nourished in the normal way. On the addition of sympathetic extract to the blood passing through the brain no fall in the systemic blood pressure took place. That the vasomotor centre was in an active condition was shown by the fact that the systemic blood pressure rose considerably when the circulation to the brain was shut off (asphyxia.)

Similar experiments were done on two other cats.

It follows from these perfusions that the fall in blood pressure is not a bulbar action. It will now be demonstrated that the fall is possible in the absence of the bulb.

Exclusion of vasomotor centre in bulb. — In this experiment the vessels going to and coming from the head were ligated in a manner similar to that described in the preceding experiment. The cannula of a syringe filled with sympathetic extract was tied into the femoral vein and the systemic blood pressure was taken from the left carotid artery. Both vagi were divided. The posterior arches of the second and third cervical vertebræ were removed and the spinal cord cut immediately below the bulb. The blood pressure at once fell from 105 to 40 mm. Hg. The distal end of the cord was now stimulated with rapidly repeated induction shocks in order to bring the blood pressure once more to a level at which the lowering action of the extract might be evident; the blood pressure rose to 80 mm. Hg and remained constant at this level. The sympathetic extract was injected into the femoral vein during the stimulation of the cord. At once a decided fall (between 20 and 30 mm. Hg) in the blood pressure took place. Six injections were made in this same animal and all gave similar results.

Having thus excluded the bulbar centre, we must next inquire whether the extract secures its results by means of the spinal vasomotor centres. To answer this question perfusions were made in tissues and organs isolated entirely from the central nervous system.

Perfusion of the isolated kidney. — A small dog was anæsthetized with morphia and ether and the abdomen opened in the median line. The renal artery and vein were then ligated separately close to their respective origins and a glass cannula filled with 0.8 per cent sodium chloride solution inserted in each. The kidney was carefully freed from its bed and placed in a Roy[1] oncometer which was connected with an Ellis[2] piston recorder writing on the smoked surface of a revolving drum. The cannula in the renal artery was connected with the perfusion apparatus and the normal perfusion fluid consisting of defibrinated dog's blood diluted one half with 0.8 per cent sodium chloride solution was forced through the kidney at a constant pressure of 115 mm. Hg. An assistant held the two cannulas in position, maintaining them in a straight line with the vessels and so avoided any possibility of the vessels twisting or kinking and consequently impeding the blood flow through the organ. The perfusion apparatus was so arranged that blood containing sympathetic ganglia extract could be passed from a second reservoir through the kidney as desired in place of the normal perfusion fluid. This change could be effected without altering the pressure in the perfusion tubes. The perfusion fluids were maintained at a constant temperature (37.5°C.). A glass tube conveyed the blood from the cannula in the renal vein to a beaker, and here the outflow was measured by counting the number of drops falling from the tube in successive periods of thirty seconds. That the perfusion of blood containing the sympathetic extract markedly increased the flow from the renal vein can readily be seen by studying Figure 2. So great was this increase that in three of the thirty second periods the blood flowed from the renal vein in a stream and counting became impossible. The curve in Figure 2 is therefore interrupted, blank spaces representing the period during which the outflow was too rapid to be counted.

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[1] Roy and Cohnheim: Untersuchungen über die Circulation in den Nieren, Berlin, 1883.

[2] Ellis: Journal of physiology, 1886, iv, p. 309.

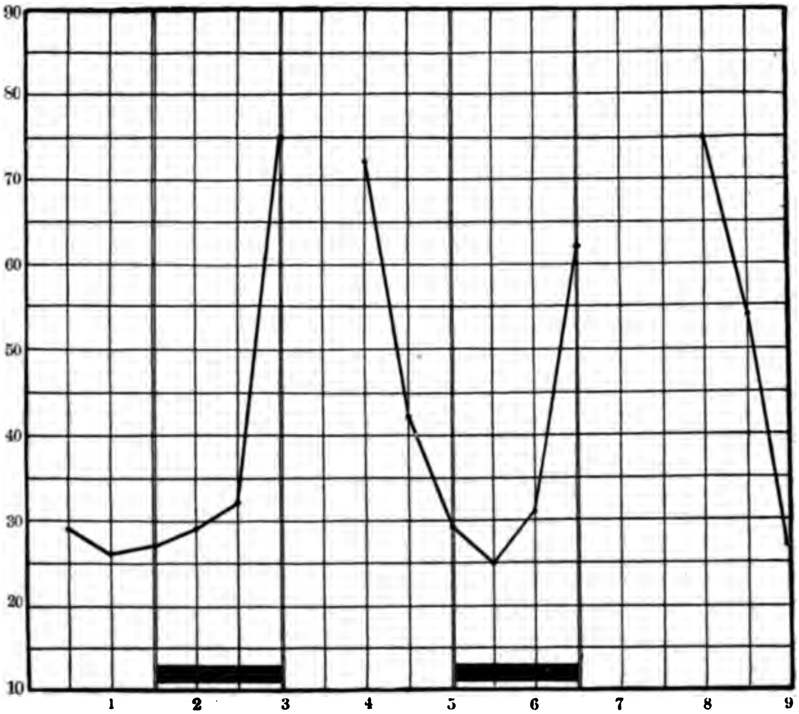

FIGURE 2. Curve showing the increased outflow from the renal vein in consequence of the perfusion of sympathetic extract. During the breaks in the curve the outflow was too rapid to be counted. The heavy black lines mark the duration of the perfusion. The ordinates give the number of drops; the abscissæ, intervals of 30 seconds.

Further evidence was obtained by the perfusion of the submaxillary gland.

Perfusion of submaxillary gland. — A medium size dog was anæsthetized with morphia and ether, tracheotomized, and the artery and veins of the submaxillary gland laid bare. A glass cannula was then placed in the duct of the gland, another in the facial artery just before it gives off the submaxillary branch, and another in the external jugular vein. The facial artery was ligated above and below the origin of the branch to the gland; all tributaries of the external jugular vein were also ligated except those coming direct from the gland, and a small artery which enters the gland on its dorsal margin was tied. Thus the gland was isolated entirely from the systemic circulation. It will be noticed that the nerves going to the gland were left intact. The cannula in the facial artery was now connected with a perfusion apparatus and defibrinated dog's blood at body temperature was passed through the gland at a pressure of 100 mm. Hg.

In three experiments the amount of blood issuing from the cannula in the jugular vein was measured during successive periods of two minutes, while the gland was being perfused with normal blood, and with blood containing sympathetic extract. When the gland was perfused with blood containing the sympathetic extract, the outflow was increased 21.4 per cent, 25 per cent, and 71.4 per cent in the three experiments respectively. Two other dogs gave a similar result.

Perfusion of hind limb. — A dog received a small dose of morphia, and was then kept fully under the influence of ether. The superficial femoral artery and vein of the right hind limb were exposed. The artery was ligated above the origin of the profunda femoris and the vein above the similar venous branch. Glass cannulas were inserted into the artery and vein below the profunda artery and vein. This was done in order to abolish the free anastomosis existing through these vessels between the superior and inferior regions of the thigh, through which channels blood perfused into the limb might escape into the general circulation. The perfusion of the limb was carried out in a manner similar to that already described for the kidney experiments. A blood tracing from the carotid artery was recorded on a drum. It was found that the outflow from the femoral vein (75 c.c.) during four minutes' perfusion with the sympathetic extract was exactly double the amount issuing from the vein during the perfusion of normal blood in the same length of time. No change took place in the systemic blood pressure.

It appears from these several experiments that marked dilatation of the blood vessels follows the perfusion of parts isolated from the central nervous system and hence cut off from communication with the vasomotor centres. The dilatation is so great as to make it probable that the action of the extract is chiefly on the sympathetic vasomotor neurons or on the blood vessels themselves. The following experiments strengthen the latter opinion by showing that the extract does indeed affect the contractility of muscle.

Cardiac muscle; the "apex" of the dog's heart. — The technique and apparatus employed in this experiment I have described at length in a former publication.[1]

A cannula was tied in one of the apical divisions of the ramus descendens of the left coronary artery and the part of the apical half of the ventricle supplied through the arterial branch cut out (Porter's method).[2] The excised apex was placed in a wide glass tube inserted in a large tank of water kept at a constant temperature of about 36.5° C. The cannula was connected to perfusion flasks also immersed in the tank, and the preparation perfused at a constant pressure of about 100 mm. Hg with defibrinated dog's blood diluted with an equal volume of 0.8 per cent sodium chloride solution. The contractions were registered by means of a fine wire leading from a hook in the apex to an ordinary muscle lever magnifying seven times. Three dogs were used and two apex preparations were made from each heart. In all six cases the results were identical. When the normal blood passing through the apex was replaced by blood containing the extract of sympathetic ganglia there was at once a great difference in the character of the contractions. Strong doses of the extract (20 per cent) slowed the contractions and diminished their force while at the same time a very considerable fall in tonus was produced. Smaller doses — from 5 to 10 per cent — did not seem to affect the rate of the beat but increased considerably the height of the contractions

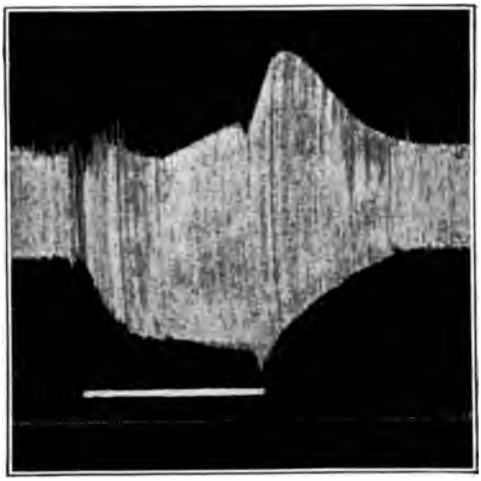

FIGURE 3. Original size. Curve drawn by lever attached to "apex" of perfused dog's heart. Magnification × 7. Time in periods of 5 seconds. During the interval marked by the white band, blood containing sympathetic extract was substituted for the normal perfusion blood.

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[1] CLEGHORN: This journal, 1899, ii. p. 273.

[2] PORTER: Journal of experimental medicine, 1897, ii. p. 391.

and caused a marked fall in tonus. The fall in tonus compensated for the increased contraction, that is to say although the amplitude of the beats was increased the fall in tonus was sufficient to prevent the top of the contraction curve from rising above the normal.

Skeletal muscle. — The action of the extract on skeletal muscle was tested on the gastrocnemius muscle of frogs in which from 1 to 2 c.c. had been injected into the dorsal lymph-sac.

About ten minutes after the injection the animal showed signs of distress and the respiration became labored and quickened. In fifteen or twenty minutes the hind legs were extended. Frequently the animal would be found in this state on its back, from which position it could only recover itself with difficulty. When placed on the floor and stimulated to jump the frog could not jump straight but always turned more to one side or the other, and the jump at best appeared jerky although strong. In two cases the frogs were found dead twenty minutes after the injection. Both were in the dorsal position with their hind legs powerfully extended. In the four remaining frogs the effect passed off in about forty-five minutes.

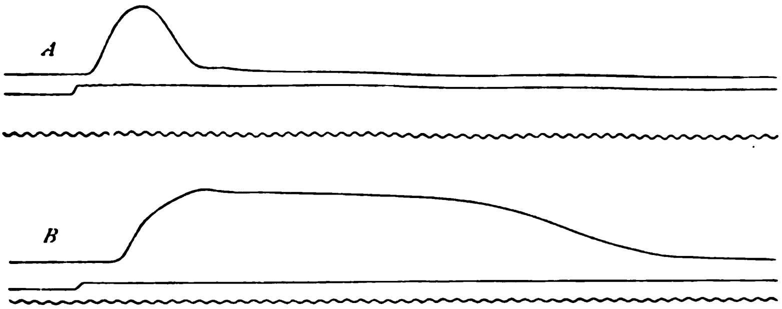

FIGURE 4. Two thirds the original size. Curves drawn by gastrocnemii muscles, *A*, normal; *B*, poisoned by the injection of sympathetic extract into the dorsal lymph-sac. The rise in the middle line indicates stimulation with maximum break shock. The lowest curve gives the time in $\frac{1}{100}$ seconds. The levers magnified 9 times.

A preparation of the sciatic nerve and gastrocnemius muscle was made from each of six poisoned frogs and from the same number of normal animals and the contractions of the several muscles in response to maximum break shocks were recorded on a Baltzar drum revolving at a rapid speed. A great difference in the character of the contractions may be noticed (see Fig. 4). The latent period of the poisoned muscle is half again as long as in the normal muscle preparation. The actual contraction or shortening of the muscle is also prolonged while the period of relaxation is extended to such a degree as to resemble greatly the curve of a muscle poisoned with veratrine.

It is evident from the experiments recorded in this study of the action of the extract of sympathetic ganglia on the circulation that the fall in blood pressure is chiefly, or wholly, peripheral; in other words is dependent on either the sympathetic vasomotor cells, or the direct dilation of the vessels themselves, or, possibly, on both these mechanisms. Evidence has been adduced which makes it somewhat probable that the extract acts on the blood vessels without the mediation of the sympathetic system, but this at present must not be affirmed with too much confidence.

ACTION ON THE ISOLATED HEART, AND ON THE PUPIL.

The isolated heart. — The tortoise heart was perfused with defibrinated cat's blood, diluted one half with 0.8 per cent sodium chloride solution, through the ventricular coronary system in the manner described by Gaskell.[1] Three preparations were used. The circulation was so slow — even with 100 mm. Hg pressure — that the results do not stand out with the distinctness noticed in the dog's apex. In all the experiments however the extract produced a slowing of the rate and a slight lessening in the force of the contraction and in the tonus.

In three experiments the heart of the frog was removed from normal animals, with all its vessels ligated, and its contractions recorded by means of a light lever attached to the apex. Injections of sympathetic extract were made into the bulbus arteriosus by means of a hypodermic syringe with a very fine needle. In all cases the heart stopped beating and a very slight fall in tonus took place. After a few minutes recovery as a rule occurred. Injection of normal saline solution as a control experiment stimulated the heart, causing an accelerated and more powerful action.

Pupil. — In no experiment was any alteration in the pupil noticed either when the extract was intravenously injected or applied to the conjunctive directly.

In conclusion I desire to acknowledge with thanks the valuable assistance of Prof. W. T. Porter, and also to thank Dr. Albert P. Mathews for his kind aid in the operations on the submaxillary gland.

SUMMARY.

1. The injection of glycerine or aqueous extract of the semilunar ganglia of the ox, sheep, and pig, and of the superior and inferior

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[1] GASKELL: Journal of physiology, 1883, iv, p. 43.

cervical, stellate, and semilunar ganglia of the dog and cat causes almost immediately a marked temporary lowering in blood pressure.

2. The fall in blood pressure caused by "sympathetic extract" can be checked or counteracted by the injection of extract of the suprarenal bodies, and *vice versa*.
3. The lowering of the blood pressure is not brought about through the vasomotor centres but by the action of the extract on the blood vessels themselves, or possibly on their sympathetic vasomotor neurons.
4. Perfusion with the extract lowers the tonus of the heart muscle.
5. The latent period and the phase of relaxation of skeletal muscle is lengthened.
6. No action on the pupil was observed.

THE DIASTATIC ACTION OF PANCREATIC JUICE.

By B. K. RACHFORD.

IN recent years many observers have devoted much time and labor to the study of the action of unorganized ferments on food stuffs. In this study amylopsin, the diastatic ferment of the pancreas, has been almost wholly neglected. In fact, as far as I am aware no systematic study of the action of amylopsin on starch under the various conditions herein described has ever been made with pure pancreatic juice.[1]

Chittenden, Langley and Eves, and others have given us most valuable information on the diastatic action of saliva as modified by various conditions, suggested by the conditions under which diastatic ferments are supposed to act in the digestive tract; and in the absence of experimental work to determine the diastatic action of pancreatic juice, under such varying conditions, we have for the most part been forced to formulate our knowledge of the action of amylopsin from the experimental work done with ptyalin and other diastatic ferments. It may be that the diastatic action of the pancreatic juice will under all conditions be the same as that of saliva; yet, however this may be, there can be no doubt that it would be more desirable to have our knowledge of the diastatic action of pancreatic juice based upon experiments made with the juice itself rather than be forced to infer the extent of its action under diverse conditions from the action of other diastatic ferments under similar conditions.

In this statement I do not wish to appear to underrate the value of these studies or to insist that the principles of starch digestion, as obtained from the study of the diastatic action of saliva and malt, shall not be applied in explaining the diastatic action of pancreatic juice in the small intestine, but it goes without saying that confirmatory experiments made with pure pancreatic juice would substitute certainty for inference and place our knowledge of the intestinal digestion of starches on a much more satisfactory footing.

The work of pancreatic juice in carrying on the intestinal digestion of starchy foods is recognized as a digestive function second in importance to none. That experimenters have neglected this field of work is not therefore due to its unimportance, but rather to the difficulties in the way of obtaining pure pancreatic juice in quantities sufficient to carry on a systematic research. The purpose therefore of this paper is not only to record some experiments, which I recently have made, but also and more especially to call attention to a *method* of obtaining pure pancreatic juice from the rabbit in sufficient quantity for experimental purposes.

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[1] See the criticism of HAMBURGER: Archiv f. d. ges. Physiol., 1895, lx, p. 558.

Operation for temporary pancreatic fistula in the rabbit. — The operation for making a temporary pancreatic fistula in the rabbit was first described by me in the Journal of physiology, 1891, xii, p. 80. The operation given here in detail is an improvement on the original and makes it possible for any observer to obtain sufficient pancreatic juice for research. Experience has taught me that it is a waste of time to use any but the largest and strongest rabbits. With such animals the operation for temporary pancreatic fistula is easily and quickly done as follows. After removing the hair make an abdominal incision in the linea alba, on a level with the lower ribs, three centimetres long. Find the duodenum, which is easily reached high up in the right hypochondriac region lying close against the abdominal wall, and bring it through this opening. Run down the gut to a point where the peritoneum binds it so closely that it will not come through the opening without tearing the mesenteric attachment, and just at this point will be found the pancreatic duct as it passes through a leaf of the pancreas to enter the intestinal canal. Gently separate the mesenteric attachment of the gut so as to bring the latter through the abdominal opening without tearing blood vessels and producing unnecessary hemorrhage. By holding the gut to the light the pancreatic duct can readily be observed and two points chosen, one on either side about two centimetres from the papilla, for applying ligatures to the intestine. These points should be selected with an eye to the vascular distribution to the intestine, so that the blood-supply shall be disturbed as little as possible. The ligatures are tied at this point so as to occlude the lumen of the intestine; the ends are then passed around the body of the animal and tied in that position. If it be preferred small clamps, resembling prepuce clamps, may be used instead of ligatures. All of the intestines except that portion included between the clamps or ligatures is returned to the abdominal cavity *without section.* This arrangement will maintain the relative position of the parts, so that the portion of the mesentery holding the pancreatic duct cannot be disarranged by peristaltic contractions or by the subsequent movements of the animal. If a large abdominal wound is made it is sometimes necessary to close it partially by stitches, so that the intestine cannot be forced through the opening and in that way disarrange the parts. Care must be taken however that the abdominal wound be sufficiently open to permit the free passage of the mesentery carrying the pancreatic duct. The portion of the intestine included between the clamps or ligatures is now laid open opposite its mesenteric attachment and spread out on the abdominal wall. The lateral margins are packed with absorbent cotton to prevent bleeding. The pancreatic papilla can be observed on the exposed mucous membrane. In a short time it will open and pancreatic juice will exude. Into this papilla is inserted a small glass cannula to which has been attached an inch or two of rubber tubing. The cannula being in position, the exposed mucous membrane is covered with absorbent cotton, which may if necessary be saturated from time to time with warm physiological salt solution. The flow of juice may begin at once, or it may not commence for an hour after the insertion of the tube. When the pancreatic juice commences to flow it continues from five to eight hours. During this time the juice is collected by emptying the filled cannula from time to time and reinserting it. In this manner may be obtained from three to five c.c. of pancreatic juice uniform and powerful in physiological action. It is my custom to have two rabbits under operation at the same time, and in this way I never fail to obtain juice sufficient for an experiment. The pancreatic juice collected from both rabbits is mixed together in a single tube and afterward divided equally between the tubes containing the digestive mixtures. In this way one is sure that all the tubes of a single experiment contain pancreatic juice uniform in physiological action.

Influence of bile and hydrochloric acid on the diastatic action of pancreatic juice. — In the starch experiments here recorded the bile used was obtained from the same rabbits that furnished the pancreatic juice.

The method used in determining the rate of diastatic action was the one described by Pavy[1] under the title "Quantitative determination of sugar by the ammoniated cupric test."

The time of each experiment was 45 minutes. When the diastatic action was stopped by boiling the mixtures, the total volume of liquid in each tube was 60 c.c. One gram of wheat starch boiled in a definite quantity of water was used in each.

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[1] PAVY, F. W.: Physiology of the carbohydrates, London, 1894.

The hydrochloric acid used in these experiments was in the form of a 0.1 per cent solution. I made a number of tests to determine the amount of this acid solution necessary to neutralize a given quantity of pancreatic juice and the results of these tests are noted elsewhere in this paper. Determinations were also made by Dr. W. H. Crane, who was given 5 minims of pure pancreatic juice and a sample of the 0.1 per cent hydrochloric acid solution used in the experiments, with the request that he estimate the amount of the acid solution necessary to neutralize the 5 minims of juice.

Dr. Crane diluted the pancreatic juice with 5 c.c. of distilled water and added a drop of 0.5 per cent alcoholic solution of dimethyl amido azo-benzol. Ten c.c. of 0.1 per cent hydrochloric acid were diluted to 100 c.c., placed in a burette, and added drop by drop to the pancreatic juice. Neutralization was obtained with 4.5 c.c. of the 0.01 per cent hydrochloric acid. Thus 5 minims of pancreatic juice required 0 45 c.c. of 0.1 per cent hydrochloric acid for neutralization.

It may here also be noted that all the hydrochloric acid tubes, in the above or in subsequent experiments, contained from 2 to 10 c.c. of the hydrochloric acid solution in excess of the amount required to neutralize the pancreatic juice, and that the subsequent addition of bile or albumin was not sufficient to neutralize this excess of free acid. All hydrochloric acid tubes therefore in the following experiments except those to which sodium carbonate was added contained free acid.

I submitted to Dr. Crane a fluid containing the following ingredients: pancreatic juice 10 minims, 0.1 per cent hydrochloric acid solution 3 c.c., starch 1 gram, water 25 c.c., with the request that he determine whether or not the fluid contained free acid, and if acid how much bile would be required to neutralize the acid.

Dr. Crane found 27.4 c.c. of fluid in the test tube. It was acid to dimethyl amido azo-benzol. Of the starchy fluid 13.7 c.c. were placed in a beaker and bile (0.9 c.c. of which had been diluted to 9.0 c.c. with water) introduced from a graduated pipette. Neutralization required 6.2 c.c. Thus 27.4 c.c. of the fluid are neutralized by 1.24 c.c. bile.

I would call attention to the fact that the mixture upon which the above report was made was taken as a sample of the hydrochloric acid tubes in the experiments on page 487. It must be noted, however,

Experiment.	Pancreatic juice.	Water.	0.1 per cent HCl.	Bile.	Starch.	Starch converted.
I	minims.	c.c.	c.c.	minims.	gram.	Per cent.
1	10	60	0	4	1	30
2	10	57	3	4	1	27
3	10	57	3	0	1	23
4	10	60	0	0	1	30
II						
1	10	60	0	8	1	50
2	10	57	3	8	1	50
3	10	57	3	0	1	37
4	10	60	0	0	1	40
III						
1	10	60	0	6	1	32
2	10	57	3	6	1	32
3	10	57	3	0	1	20
4	10	60	0	0	1	25
IV						
1	7	60	0	5	1	25
2	7	57	3	5	1	27
3	7	57	3	0	1	24
4	7	60	0	0	1	26
V						
1	10	60	0	5	1	34
2	10	57	3	5	1	36
3	10	57	3	0	1	30
4	10	60	0	0	1	34
VI						
1	10	60	0	8	1	34
2	10	57	3	8	1	36
3	10	57	3	0	1	24
4	10	60	0	0	1	28

that this mixture contained the minimum amount of acid used in any of the digestive mixtures, and that this mixture was found to require 1.24 c.c. of bile to neutralize it; a much larger amount of bile than was used in any of the tubes, except tubes 1 and 2 of Experiment VII.

In these tubes, however, it will be noted that the 8 and 10 c.c. of the hydrochloric acid solution which these tubes respectively contained was sufficient to give to them an acid reaction.

Studying the above experiments it is plain that bile slightly expedites the diastatic action of pancreatic juice. The expediting influence of the bile, however, is here shown to be so slight that the manner of its action scarcely merits discussion. It is important to observe that the bile does not exert an unfavorable influence on the diastatic action of pancreatic juice. But it is of much more importance to note that the 0.1 per cent hydrochloric acid solution used in these experiments had only a slight retarding influence on the diastatic action of the ferment. A slight retardation is, however, seen in every case. Tube 3 in each of the above experiments contains 3 c.c. of a 0.1 per cent solution of hydrochloric acid, which gave to the contents a decided acid reaction. In experiments made to determine the amount of the hydrochloric acid solution necessary to neutralize pancreatic juice, it was found that 12 to 15 minims of the hydrochloric acid solution would destroy the alkalinity of 10 minims of pancreatic juice and that 15 to 18 minims would give to it an acid reaction. Hence the amount of hydrochloric acid solution in these tubes was more than 2 c.c. in excess of the amount required to neutralize the alkalinity of the pancreatic juice, and yet in the presence of this excess of free hydrochloric acid the pancreatic juice had almost as much diastatic action as it had when acting alone in the presence of its own alkaline salts.

Of yet greater interest are the tubes marked 2 in the above experiments. Each of these tubes contained not only 3 c.c. of the hydrochloric acid solution, but also a certain amount of bile. A study of these tubes shows us that the bile not only neutralized the slight retarding influence of the free hydrochloric acid but also furnished the conditions in the presence of this acid for pancreatic juice to do its most rapid diastatic work. The tubes containing both bile and pancreatic juice, in every experiment except one, lead in the amount of diastatic work done. In the experiment in which the bile and hydrochloric acid tube fails to do the most work, the failure is probably due to the small amount of bile used. Whatever may be the explanation, it is an important physiological fact that bile, when added to pancreatic juice acting in the presence of a small quantity of free hydrochloric acid, will not only neutralize the retarding influence which the free acid has on the diastatic action of pancreatic juice, but will in doing so furnish the most favorable conditions for the action of this ferment. And it is also important to note that bile will accomplish this result without neutralizing the acid completely, thus showing that the favorable influence of the bile on the diastatic action of pancreatic juice is not simply one of acid neutralization.

The following experiment shows even more clearly the value of bile when pancreatic juice is acting in the presence of free hydrochloric acid. Here the retarding influence of 8 and 12 c.c. of a 0.1 per cent hydrochloric acid solution is very marked, but in tube 1, containing 8 c.c. of the hydrochloric acid solution, twenty minims of bile not only neutralizes the retarding action of the hydrochloric acid but also enables the pancreatic juice to do even more diastatic work than is done in tube 5, where the pancreatic juice is acting apart from the influence of either hydrochloric acid or bile. In tube 2, 30 minims of bile enables 12 minims of pancreatic juice acting in the presence of 12 c.c. of hydrochloric acid solution to digest 24 per cent of starch instead of 12 per cent, the amount digested in tube 3 in which the same quantity of pancreatic juice acted in the presence of 12 c.c. of the hydrochloric acid solution without the assistance of the bile.

EXPERIMENT VII.

	Pancreatic juice.	Water.	0.1 per cent HCl.	Bile.	Starch.	Starch reduced.
	minims.	c c.	c.c.	minims.	gram.	Per cent.
1	12	52	8	20	1	40
2	12	48	12	30	1	24
3	12	48	12	0	1	12
4	12	52	8	0	1	14
5	12	60	0	0	1	30

In Experiment VIII, even more graphically than in any that has preceded it, is shown the retarding influence of free hydrochloric acid on the diastatic action of pancreatic juice and the value of bile in neutralizing this retarding action. Here 12 c.c. of the hydrochloric acid solution almost destroys the diastatic action of 15 minims of pancreatic juice, and 8 c.c. of the acid solution greatly retards its action, while 7 minims of bile is sufficient to neutralize entirely the retarding influence of 8 c.c. of the acid solution.

EXPERIMENT VIII.

	Pancreatic juice.	Water.	0.1 per cent HCl.	Bile.	Starch.	Starch reduced.
	minims.	c.c.	c.c.	minims.	gram.	Per cent.
1	15	60	0	0	1	25
2	15	52	8	0	1	14
3	15	48	12	0	1	2
4	15	52	8	7	1	26
5	15	48	12	7	1	8
6	15	52	8	0	1	18
7	15	48	12	0	1	5
8	15	52	8	7	1	25
9	15	48	12	7	1	6

The preceding experiments clearly establish the fact that bile is not necessary to the diastatic action of pancreatic juice acting alone uninfluenced by hydrochloric acid, but that bile may be of the greatest assistance to pancreatic juice, indeed almost necessary to its full diastatic action, when the former is acting in the presence of free hydrochloric acid, and that bile can serve this purpose without neutralizing all of the free acid present.

The influence of acid albumin on diastatic action of pancreatic juice. — In Experiment IX, the egg albumin was mixed with the hydrochloric acid solution before the pancreatic juice was added to the tube, and here it will be seen that 0.3 gram of egg albumin exer-

EXPERIMENT IX.

	Pancreatic juice.	Water.	0.1 per cent HCl.	Egg albumin.	Bile.	Starch.	Starch reduced.
	minims.	c.c.	c.c.	gram.	minims.	gram.	Per cent.
1	10	60	0	0	0	1	23
2	10	56	4	0.3	0	1	31
3	10	52	8	0.3	0	1	19
4	10	56	4	0.3	0	1	37
5	10	52	8	0.3	0	1	15
6	10	56	4	0.3	10	1	38
7	10	52	8	0.3	10	1	25
8	10	60	0	0	10	1	25

cised very much the same influence on pancreatic juice acting in the presence of free hydrochloric acid that the bile did in the previous experiments. Tubes 2, 4, and 6 demonstrate that acid albumin very materially expedites the diastatic action of pancreatic juice. Tubes 6, 7, and 8 of this experiment contain each 10 minims of bile. In tube 6 the presence of the bile seems to add little to the diastatic action of the pancreatic juice since almost the same percentage of starch is digested in tubes 2 and 4, which do not contain bile, but in other respects resemble tube 6. In tube 7, however, we learn by comparison with tubes 3 and 5 that the 10 minims of bile had a very decided influence in increasing the diastatic action of the pancreatic juice in the conditions under which it is acting. In tube 7 of this experiment 0.3 gram of egg albumin was not sufficient to neutralize fully the retarding action of 8 c.c. of the hydrochloric acid solution; the addition of 10 minims of bile, however, accomplished this. The failure of bile to increase further the diastatic action of pancreatic juice in tube 6 is due to the fact that the 0.3 gram of egg albumin is almost if not quite sufficient to neutralize the 4 c.c. of hydrochloric acid solution, and for this reason the further addition of bile or egg albumin would not materially increase the action of the pancreatic juice.

Influence of sodium carbonate on the diastatic action of pancreatic juice. — Experiment X was planned for the purpose of studying the influence of sodium carbonate on the diastatic action of pancreatic juice acting under various conditions. A one per cent solution of sodium carbonate was used. Tubes 2 and 3 of this experiment show that 2 and 5 c.c. of the sodium carbonate solution almost entirely destroy the diastatic action of 5 minims of pancreatic juice; while tubes 4 and 5 show that 5 minims of bile have a decided influence in neutralizing the retarding influence which the soda solution has upon the diastatic action of pancreatic juice. Tubes 6 and 7 indicate that the 0.6 gram of egg albumin also had some influence in neutralizing the retarding action of the soda.

EXPERIMENT X.

	Pancreatic juice.	Water.	1 per cent Na_2CO_3.	Bile.	Egg albumin.	0.1 per cent HCl.	Starch.	Starch reduced.
	minims.	c.c.	c.c.	minims.	gram.	c.c.	gram.	Per cent.
1	5	60	0	0	0	0	1	50
2	5	58	2	0	0	0	1	7
3	5	55	5	0	0	0	1	2
4	5	58	2	5	0	0	1	15
5	5	55	5	5	0	0	1	6
6	5	58	2	0	06	0	1	10
7	5	55	5	0	0.6	0	1	4
8	5	52	5	0	0.6	3	1	13

If hydrochloric acid be added to a mixture in which pancreatic juice is acting on starch in the presence of sodium carbonate the acid will neutralize the retarding influence of the alkali. This is shown in tube 8. This action may be simply one of chemical neutralization, but whatever the explanation, the observation is interesting as showing that the diastatic power of pancreatic juice in a strongly alkaline mixture may be increased by the addition of a small quantity of hydrochloric acid.

EXPERIMENT XI.

	Pancreatic juice.	Water.	0.1 per cent Na_2CO_3.	Bile.	Starch.	Starch reduced.
	minims.	c.c.	c.c.	minims.	gram.	Per cent.
1	4	60	0	0	1	50
2	4	58	2	0	1	5
3	4	55	5	0	1	3
4	4	58	2	4	1	11
5	4	55	5	4	1	13
6	..	60	0	4	1	8
7	..	60	0	4	1	8

Experiment XI again demonstrates the destructive action of sodium carbonate on the diastatic action of pancreatic juice, and also shows the value of bile in neutralizing this retarding influence. In tubes 6 and 7 it is shown that bile itself has some diastatic power.

CONCLUSIONS.

(1) A small quantity of free hydrochloric acid has little or no retarding influence on the diastatic action of pancreatic juice.

(2) Larger quantities of free hydrochloric acid very materially retard the diastatic action of pancreatic juice; in one experiment 12 c.c. of a 0.1 per cent solution of hydrochloric acid almost destroyed the diastatic action of 15 minims of pancreatic juice acting in a mixture of sixty cubic centimetres volume; in another experiment the same quantity of acid reduced by two thirds the diastatic action of 12 minims of pancreatic juice. The facts that different specimens of pancreatic juice vary in their degree of alkalinity and in their diastatic power make it impossible to formulate precise statements concerning the influence which definite quantities of acid will have on definite quantities of pancreatic juice.

(3) Acid proteids in small quantities slightly increase the diastatic action of pancreatic juice. Neutral proteids therefore when added to pancreatic juice acting in the presence of free hydrochloric acid

will not only neutralize the retarding action of the acid on the diastatic action of the juice, but they will also by the formation of acid albumin assist materially the pancreatic juice in its work.

(4) Sodium carbonate has a very destructive influence on the diastatic action of pancreatic juice. Two cubic centimetres of a one per cent solution of sodium carbonate almost totally destroys the diastatic action of 5 minims of pancreatic juice acting in a mixture of sixty cubic centimetres volume.

(5) Bile has no retarding influence on the diastatic action of pancreatic juice; in fact it slightly expedites its action.

(6) Bile not only neutralizes the retarding influence which free hydrochloric acid has upon the diastatic action of pancreatic juice, but in the presence of free hydrochloric acid it very materially expedites the action of the juice. Here again it is impossible to formulate rules as to the exact amount of bile necessary to neutralize the retarding influence of a definite quantity of hydrochloric acid, and thus give to a definite quantity of pancreatic juice its greatest diastatic power. In the above experiments, however, it will be seen that four to eight minims of bile were sufficient to neutralize the retarding influence of from four to eight cubic centimetres of a 0.1 per cent hydrochloric acid solution without destroying the acid reaction of the mixture and thus to give to pancreatic juice its greatest diastatic power.

(7) Bile has a marked influence in diminishing the retarding influence which sodium carbonate has upon the diastatic action of pancreatic juice.

(8) Bile itself has some diastatic power.

Not the least interesting point in these conclusions is the suggestion that bile may play a not unimportant part in the intestinal digestion of starches. If there be any free acid in the food, as it is discharged from the stomach into the duodenum, the bile will neutralize this acid and thereby assist the acid proteids discharged with the starches through the pylorus, in furnishing the most favorable conditions for the diastatic action of pancreatic juice. And possibly of even more importance is the fact that bile will limit the destructive action of sodium carbonate on the diastatic action of amylopsin. In this connection it is interesting to note that in herbivorous or starch-eating animals[1] the bile as a rule is poured into the duodenum far below the point where the pancreatic duct enters. This arrangement would give to the bile the most favorable opportunities for neutralizing any retarding influence which the sodium carbonate of the intestinal juice might exert on the diastatic action of pancreatic juice.

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[1] Rachford: Comparative anatomy of bile and pancreatic ducts, Medicine, 1895, p. 520.

In conclusion I wish to express my thanks to Dr. Frank Southgate, who has assisted me very greatly in the arduous details of these experiments.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

ELEVENTH ANNUAL MEETING.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

ON EPINEPHRIN, THE ACTIVE CONSTITUENT OF THE SUPRARENAL CAPSULE AND ITS COMPOUNDS.

By JOHN J. ABEL.

ACTING on Hyrtl's suggestion that *epinephris* would be the best name for the suprarenal capsule, the author has given the name Epinephrin to the active principle as isolated by him.

Aside from the chemical and physiological interest attaching to this substance it is believed that its careful study will throw light on the symptoms of Addison's disease.

When the benzoate of epinephrin is decomposed in the autoclave at pressures varying from 8 to 12 atmospheres, the resulting solution contains epinephrin; it no longer gives a rose-red color with iodine water and ammonia, but gives instead the fine emerald green which is always seen when ferric salts are added. All other reactions of epinephrin described in previous articles[1] are retained. The salts of epinephrin secured in this way possess but little physiological activity owing to some slight change, perhaps the gain or loss of a molecule of water, or some shifting of atomic groups in the molecule.

The analytical results for the free base and for some of its salts are given below in abstract. These results have led to the adoption of the formula $C_{17}H_{15}NO_4$ as expressing the composition of the new alkaloid.

Found *Epinephrin* $C_{17}H_{15}NO_4$	*Theory for* $C_{17}H_{15}NO_4$ *requires*	*Found* *Epinephrin Diacetate.* $C_{17}H_{15}NO_4(CH_3.COOH)_2$	*Theory for* $C_{17}H_{15}NO_4(CH_3.COOH)_2$ *requires*
C = 69.12	C = 68.68	C = 58.16	C = 60.43
H = 5.24	H = 5.05	H = 5.82	H = 5.51
N = 5.00	N = 4.71	N = 5.04	N = 3.36

The salt lost acetic acid on drying and showed signs of decomposing.

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[1] ABEL: Johns Hopkins hospital bulletin, July, 1897, and September-October, 1898.

Epinephrin Benzoate $C_{17}H_{14}NO_4 . CO . C_6H_5$	*Theory for* $C_{17}H_{14}NO_4 . CO . C_6H_5$ *requires*	*Epinephrin Picrate* $C_{17}H_{15}NO_4 . C_6H_2 . (NO_2)_3 . OH$	*Theory for* $C_{17}H_{15}NO_4 . C_6H_2 . (NO_2)_3 . OH$ *requires*
C = 72.54	C = 71.82	C = 52.29	C = 52.47
H = 5.54	H = 4.74	H = 4.03	H = 3.42
N = 3.46	N = 3.49	N = 10.54	N = 10.65

Epinephrin sulphate $C_{17}H_{15}NO_4 . H_2SO_4$	*Theory for Epinephrin sulphate* $C_{17}H_{15}NO_4 . H_2SO_4$ *requires*
Sulphuric acid found = 24.80%	H_2SO_4 = 24.78%

The sulphate was made from the picrate, which had been made in turn from the free base. Both the sulphate and picrate are microcrystalline compounds, and like the benzoate they leave no ash when incinerated.

ON THE FORMATION AND COMPOSITION OF HIGHLY ACTIVE SALTS OF EPINEPHRIN.

BY JOHN J. ABEL.

WHEN the benzoate prepared directly from aqueous extracts of the glands is decomposed in the autoclave in the presence of a 1 or 2 per cent solution of sulphuric acid and at pressures varying from 3 to 5 atmospheres, intensely active solutions of epinephrin are obtained. Such solutions also retain the property common to aqueous extracts of the gland, of giving a rose-red color when treated with iodine water and ammonia. If epinephrin is precipitated out of such solutions, immediately dissolved in dilute acids and tested on animals, it is found to possess considerable physiological activity. Nevertheless the chemical operations involved in the formation of salts from the free base, such as prolonged washing with water and exposure to air, so greatly reduce their physiological activity that it was determined to form the picrate by direct precipitation with sodium picrate immediately after removing the hydrolyzed benzoate from the autoclave. The picrate obtained in this way was highly active, although somewhat contaminated with a picrate of unknown composition and of a very high nitrogen content. This active picrate and the sulphate made from it give the rose-red color with iodine water already mentioned. The active but impure picrate has the following composition: —

$C = 50.13$ $H = 4.02$ $N = 13.02$

A sulphate [A], also not quite pure, but highly active, yielded on analysis 22.20 per cent sulphuric acid. A second sulphate [B] equally active yielded 22.88 per cent sulphuric acid. For $C_{17}H_{15}NO_4.H_2SO_4$ theory requires 24.78 per cent H_2SO_4.

Of sulphate [A] it required 0.00013 gram to raise the arterial pressure about 14 mm. Hg in a small dog. This quantity of the sulphate corresponds to 0.00009 gram of the free base. Of a third sulphate it required only 0.00011 gram to elevate the blood pressure 16 mm. Hg in a dog weighing 6.08 kilos. In other words a very distinct physiological effect is produced by so small a quantity of the sulphate as 0.000018 gram per kilogramme of body weight, or 0.000013 gram per kilogramme of the free base. A maximal and prolonged rise of arterial pressure followed on the injection of five or six times this amount. There can be but little doubt that the active sulphate either has the same composition as the inactive sulphate described and analysed, or that it differs only very little from it. On drying the highly active sulphate in the hot air bath at 110°C. for some hours its activity is almost destroyed.

An account was also given of the behavior of epinephrin toward alkaloidal reagents, toward fusion with alkalis, etc., and valid reasons were advanced for classing it with the alkaloids.

THE ACTION OF SUPRARENAL EXTRACT ON THE MAMMALIAN HEART.

By GEO. B. WALLACE and W. A. MOGK.

TRACINGS were taken directly from the dog's heart by a modified Roy and Adami myocardiograph. It was found that the suprarenal extract stimulates the vagus centre, thus inhibiting the heart. It produces also a direct stimulation of the heart muscle, resulting, when the vagus influence is removed, in an increase in the force and number of contractions. Accompanying the change in the heart action there occurs a rise in the systemic blood pressure, due to a contraction of the arterioles. The pressure in the pulmonary arteries, however, is not raised.

THE CHEMISTRY OF THE MELANINES.

By WALTER JONES, PH. D.

THE black hair of the horse's tail was allowed to stand several weeks in contact with concentrated hydrochloric acid, and from the product pigment-granules were isolated and purified. On fusing these granules with caustic potash a substance was obtained which had apparently lost none of the characters of a melaninic acid but was found on analysis to contain no sulphur. The failure of sulphur in the composition of the substance might suggest that an artificial choroid pigment had been made. This supposition however is untenable, for when the choroid pigment is similarly treated with caustic potash it loses all of its nitrogen while the melaninic acid in question contains 13 per cent of this element.

A study was also made of the action of oxidation agents on the sulphur-free melaninic acid. The results show very conclusively that when the oxidation occurs in an alkaline medium the pigment is easily decomposed, giving rise to carbon dioxide and ammonia. Intermediate products, however, can be obtained when the oxidation is carried on in an acid medium. Under these conditions a substance is formed which has the odor, volatility, and basic character of putrescine, but differs from this compound in the ease with which it is decomposed when heated with alkalies. The oxidation in an acid medium also gives rise to a light yellow insoluble substance which becomes darker when warmed with water until it finally changes to a pigment which is in outward appearance indistinguishable from the original melaninic acid. Its analysis, however, shows that it is a decomposition product of the latter, and that it can be represented by so simple a formula as $C_{18}H_{11}N_6O_{10}$.

ON THE SOLUTION OF MERCURY IN THE BODY-JUICES.

By A. S. CHITTENDEN. (Read by JOHN J. ABEL.)

GENERALLY speaking, metallic mercury cannot as such pass through the intact epithelium of any part of the body. In mercurial ointments some of the mercury is present in the form of salts of the fatty acids, and when such ointments are rubbed into the skin, conditions favorable to the solution of more of the mercury are met in chemical constituents of the secretion of the sebaceous glands. The oxygen of the air is no doubt a necessary accessory to these changes in the mercury. Even in the lungs the secretions of the bronchial surfaces must induce the solution of the mercury condensed on them.

Such reasoning has led many investigators to doubt that the body is capable of oxidizing mercury when it is introduced as such into the blood. To determine this point particles of pure mercury smaller than a red corpuscle were prepared by reducing alcoholic solutions of mercuric chloride with stannous chloride, and approximately 0.25 gram was injected into the femoral artery of four dogs, a solution of sodium chloride, 0.65 per cent, being used to keep the mercury in suspension. The injection was made by means of a fine hypodermic needle directly into the artery, which was clamped centrally during the operation, and the wound securely closed.

The urine and fæces were collected during six weeks. The acidulated urine was passed over coils of reduced copper gauze and the mercury determined according to the directions of Winternitz. Globules of mercury were found condensed in the receiving bulb and amalgamated with the gold foil which was placed beyond the bulb. The fæces contained no mercury.

It is therefore proved that the body juices can dissolve mercury when this element is introduced directly into the circulation by a method which entirely excludes the presence of a soluble salt of the metal. Sections of the paw of one of the animals failed to show any emboli. When sections of the lymph glands were submitted to Prof. William H. Welch he found in them numerous large megalokaryocytes, a condition suggesting parenchymatous embolism of the bone marrow. These cells most probably entered the circulation from the bone marrow and were then filtered out by the lymph glands.

ON THE PRESENCE OF CHOLIN AND NEURIN IN THE INTESTINAL CANAL DURING ITS COMPLETE OBSTRUCTION.

BY BEATTIE NESBITT, M. D.

A FOOD rich in lecithin (yolks of eggs) was fed to four dogs for two or three days, then the intestines were closed, and after two or three days more the animals were killed. The platinum double salt of cholin was prepared from the intestinal contents and purified. On analysis 0.1457 gram of this salt gave 0.0463 gram of platinum, or 31.77 per cent, whereas theory requires that this salt contain 31.64 per cent.

The presence of cholin was therefore proved.

From the intestinal contents of one dog, neurin was also obtained, though not enough for analysis. The presence of this highly toxic substance was proved by the solubilities of its platinum double salt and the crystalline character of this compound, and also by the behavior of the solutions of cholin toward the alkaloidal reagents when these solutions contained neurin.

The author also isolated a ptomaine which accompanied the cholin and neurin. The hydrochloride of this ptomaine is very soluble in water and in alcohol, and crystallizes in fine needles. The free base has a penetrating sweetish odor and is easily oxidized when exposed to the air. Both the platinum and gold salts are unstable and easily reduced compounds. The amount of this ptomaine which could be isolated did not suffice to establish its identity.

These experiments lead the author to believe that complete occlusion of the small intestine at its lower end will give rise to the occurrence of cholin, neurin, and perhaps other bases provided the food taken contains any considerable quantity of lecithin. While cholin would have to be absorbed in relatively large amounts to exert a marked toxic action on human beings, it is otherwise with neurin, which is many times more toxic in its action, and must be classed with the exceedingly active poisons. It has been shown both by the experiments of Schmidt and Weiss and also by those recorded in this abstract that the poisonous neurin may be formed from cholin by bacteria. In its physiological action neurin agrees closely with muscarin; especially to be noted here is its paralytic action on the heart and its power to increase the intestinal movements to such an extent that continual evacuations occur.

DIRECT AND REFLEX ACCELERATION OF THE MAMMALIAN HEART.

By REID HUNT.

THE experiments were performed upon dogs, cats, and rabbits; most of them, however, upon dogs. The accelerators were found almost always in a condition of tonic activity, — a tonus much more constant than that of the inhibitory nerves to the heart. The centres of the accelerators show greater resistance to the action of drugs, changes in blood pressure, etc., than do the cardio-inhibitory or even the vasomotor centres. Section of the accelerator nerves causes a prolongation of both systole and diastole; and at times, especially in cases in which the vigor of the heart is not great, cardiac irregularity. The latter seems to be due to the ventricles failing to follow all the beats of the auricles.

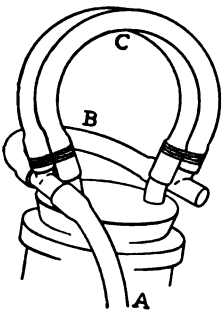
Fatigue. — When the accelerators are stimulated with an electrical current causing a maximum acceleration, the heart soon returns partially to its previous rate. At first there is a rapid decrease in the rate; then a much longer period, during which the rate remains almost unchanged or decreases very slowly. The duration of these periods depends upon the condition of the heart and nerves and upon the character and strength of the stimulus employed. The cause of the decrease in the heart rate or fatigue is a local loss of irritability of the nerve at the point stimulated; and a genuine fatigue in the heart itself. The fatigue is shown by (1) the fact that with repeated stimulations of the accelerators the heart rate becomes slower; (2) the effect upon the heart of stimulating the accelerators becomes less; (3) the effect of the vagi upon the heart becomes greater; (4) by the action of certain drugs in hastening fatigue. As drugs act upon muscle or the endings of nerves before they affect the nerve fibres, it seems probable that the very rapid decrease in the heart rate from stimulating the accelerators after certain drugs is due to their effect upon the heart rather than to their effect upon the nerve fibres. Death frequently results from repeated and long continued stimulation of the accelerators. If the vagi are in tonic activity or if they are stimulated simultaneously with the accelerators, the latter cause less fatigue of the heart; *i. e.*, the vagi have a protective influence on the heart.

Reflex acceleration. — Reflex acceleration might be caused either by increasing the activity of the accelerators or by inhibiting the tonic activity of the vagi. The latter was found to be the usual if not the exclusive method. The evidence for this is as follows: (1) When the heart is accelerated by the stimulation of a sensory nerve, the course of acceleration is much more like that resulting from the section of the vagi than that observed when the accelerators are stimulated directly; that is, the latent period is very short, the maximum acceleration is very quickly reached, and the shortening occurs mostly in diastole, whereas when the accelerators are stimulated, the latent period is long, the acceleration is slowly developed, and both systole and diastole are shortened; (2) reflex acceleration occurs just as readily after all the accelerators are divided as when they are intact; (3) in these experiments reflex acceleration was never obtained after section of the vagi.

There is some evidence for the view that there are, in most sensory nerves, two sets of fibres, stimulation of one of which causes increased activity of the cardio-inhibitory centre and so reflex slowing, while stimulation of the other set causes inhibition of this centre and so reflex acceleration.

A SIMPLE ETHERIZING BOTTLE.

By C. C. STEWART.



Two glass tubes pass through the stopper of the bottle, and by means of two T-tubes the arrangement illustrated in the diagram is procured. If the tube A be connected with the cannula in the trachea, then on clamping at B fumes of ether are inhaled; while if the clamp is placed at C, pure air may be breathed. The advantage of the arrangement is that the administration of the anæsthetic may be controlled with one hand, during either normal or artificial respiration, by changing a single clamp.

THE NATURE OF MUSCLE FATIGUE.

BY FREDERIC S. LEE.

EXPERIMENTS on the frog, the turtle, and the cat show that our previous knowledge of fatigue, limited largely to the frog, is incomplete. The essential element in the course of fatigue, common to all three species, is a decrease of lifting power. Slowing of the phase of contraction, while slight in the frog, is great in the turtle, and apparently wanting altogether in the white muscle of the cat. Slowing of the phase of relaxation, while great in the two first-named species, is also practically wanting in the white muscle of the cat.

In seeking the cause of muscle fatigue the muscles of fasting animals were compared with those in good nutritive condition but poisoned with lactic acid, potassium lactate, or sodium lactate. The contraction curve of a muscle of a fasting animal is a normal, not a fatigue, curve. That of a muscle poisoned by the substances mentioned approximates, if it is not identical with, a normal fatigue curve. The chief cause of muscle fatigue thus appears to be poisoning by fatigue substances, and not a consumption of contractile substance.

Fatigue is a state intermediate between the fresh and the exhausted states. The latter state is due to the consumption of contractile material and is a serious condition, not easily recovered from. Fatigue is not serious, can readily be done away with, and appears to be a protective phenomenon, preventing the oncoming of exhaustion.

Fatigued muscle-cells would hardly be expected to exhibit histological characteristics different from those of fresh cells. Studies of such cells under the direction of the author, and by recent cytological methods, show no visible evidences of fatigue.

OBSERVATIONS ON THE INNERVATION OF THE INTRACRANIAL VESSELS.

By G. CARL HUBER.

THE Ehrlich methylene-blue method was employed on dogs, cats, and rabbits. In the pia mater of the dog, cat, and rabbit two kinds of nerves were found, — sensory nerves and vasomotor nerves. The sensory nerves accompany the larger vessels of the circle of Willis in bundles, containing from 15 to 20 large medullated nerve fibres. These bundles of medullated nerves, in suitable preparations, may be traced in company with the arterial branches proceeding from the circle of Willis to all parts of the pia mater, of the cerebrum and cerebellum, until the arterial branches are reduced in size to vessels possessing a media consisting of two layers of muscle cells. The vessels of the choroid plexus form an exception to this statement; these medullated sensory nerves terminate, after repeated branching, in an end-brush, situated for the main part in the adventitia of the pial arteries and in the surrounding connective tissue.

This end-brush consists of numerous fine, varicose fibrils, which may often be traced for relatively long distances before terminating. The vasomotor nerves were found in the form of a perivascular plexus of non-medullated nerve fibres. This plexus was observed on all the vessels of the circle of Willis and on the arteries of the pia, varying in size from the largest found to vessels with a media composed of two layers of muscle cells. That these varicose, non-medullated nerves terminate in the muscular coat of the arteries of the pia, and may therefore be looked upon as vasomotor nerves, was determined in tissue stained in methylene blue, fixed in ammonium molybdate, sectioned, and counterstained in alum carmine, after recognizing in the unfixed tissue — under the microscope — the non-medullated, perivascular nerves above mentioned.

In the cranial dura mater, nerves similar to those above described for the pia mater were observed.

POSSIBLE AMŒBOID MOVEMENTS OF THE DENDRITIC PROCESSES OF CORTICAL NERVE CELLS.

BY C. F. HODGE (WITH H. H. GODDARD).

A NUMBER of modern neurologists attempt to explain varying psychic conditions by the extension and retraction of the cortical dendrites and their contact granules. In case the dendrites move in amœboid fashion it should be possible by preparing the brain quickly enough to catch them in the retracted and expanded state. The method adopted consisted in cutting off the head of a young animal with a single blow of a large, thin knife, and having the parts fall instantly into large dishes of Cox's solution warmed to 39° C. Three experiments have been made on pigeons, three upon hens (one experiment in each of these sets being made to compare effects of hypnotism), two on white rats, three on kittens, and three on puppies. The material from the kittens and from one of the experiments on puppies is not yet ready for study. That from the rats and from two of the experiments on puppies has yielded uniform and definite results, which confirm Demoor's and Berkley's work, and extend the former's results to include normal physiological fatigue.

The preparations from the birds do not contradict the above results but did not stain so well and were not cut in such wise as to make rigid comparison possible.

In the case of two puppies (sisters from the same litter) one killed at 4 P.M., awake, showed the cortical dendrites varicose in 31.1 per cent of the cells. The other, after sleeping an hour and ten minutes, 8.15 P.M., showed varicosity in but 8.5 per cent of the cells, but in nearly eight per cent it was only very slight; while in the waking puppy 16 per cent were markedly varicose.

In the next experiment with puppies the first was killed at 7 A.M., on waking, the other at 5.45 P.M., after being kept awake all day. This gave much more definite results. Not all parts of the cortex appeared equally affected, a point on which we hope to gain some evidence in succeeding experiments, but through large areas in this tired puppy practically all the cells showed marked varicosity of dendrites. No such areas have been found in the rested animal, the dendrites being uniformly expanded and the contact granules distinct.

A NEW CHRONOSCOPE.

By G. W. FITZ.

THIS piece of apparatus is the result of an attempt to devise a chronoscope of simple form. It consists essentially of a tube resembling a burette tube, which is entered by one short glass tube at the top and another near the bottom; the latter connects with a water reservoir for filling the apparatus, while the upper tube serves as an overflow and determines the zero level of the water column. Connected with the lower end of the tube by a short piece of rubber tubing is a valve operated by two electro-magnets, one throwing it open, the other closing it. The valve was taken from an automatic electric gas-lighting burner. The circuits are arranged so that the opening magnet is connected with the signal key of an ordinary reaction apparatus, and the closing magnet is connected by a third wire with the response key. When the signal is given, the valve is opened and the column of water falls until arrested by the closure of the valve in response to the signal. The tube is graduated by means of a falling weight so that the interval in hundredths of a second is read off at the top of the water column. This apparatus has been found to give uniform results, and thus stands as a cheap, simple form of chronoscope. It is possible to arrange the valve to work mechanically, making a still cheaper form in which the chief expense will be for the valve itself and the time of graduation. This any one can do by means of a falling weight arranged to give stated time intervals.

ON THE MAXIMUM PRODUCTION OF HIPPURIC ACID IN RABBITS.

By GRAHAM LUSK (for F. H. PARKER).

IF a quantity of benzoic acid (as a lithium salt) be fed to fasting rabbits in quantity sufficient to unite with the glycocoll formed in the animal, but not sufficient to produce toxic symptoms, a fixed ratio will exist between the hippuric acid nitrogen and the total nitrogen of the urine. This ratio, which is about 1 : 20, would indicate that 5 per cent of the nitrogen of proteid may be eliminated in the form of glycocoll. The ratio is not increased after feeding carbohydrates, nor altered after ingestion of gelatin. Gycocoll is probably a cleavage product of proteid in metabolism to the extent indicated above.

IODINE IN THE TISSUES AFTER THE ADMINISTRATION OF POTASSIUM IODIDE.

By P. A. LEVENE.

DURING the last few years there have been found in various organisms a number of normal tissue constituents containing iodine, as iodocarnein, iodokeratin, iodospongin, iodoproteid, iodofat. Some of these bodies can be obtained synthetically without great difficulty. It was the aim of the author to investigate the tissues of the higher animals as to their power of binding iodine intramolecularly.

For this study hens were employed, since in them the chemical changes of at least one tissue could be followed from day to day. The eggs were examined from time to time during ten weeks, at the end of which period the other tissues were analysed. During the first three weeks, the author found iodine only in the form of potassium or sodium iodide. Beginning with the fourth week the iodine appeared as an organic compound, apparently as iodofat. Of the other tissues or organs, the following were examined: nervous tissues, muscular tissues, grandular organs, gastro-intestinal tract, skin, and adipose tissue. In none of them could any appreciable amount of organic iodine be detected with the exception of the bones, where a noticeable quantity of iodofat was present.

Comparing these results with all that is known on the subject, the author comes to the conclusion that only certain keratins, such as that of the hair, are capable of binding iodine in the organism; only certain proteids, such as that of the thyroid gland, have the same faculty; and only certain fats act in the same way. Whether this result depends on the peculiar chemical composition of those compounds or on a peculiar activity of the different organs or organisms the author will endeavor to solve.

CORRELATION OF THE FUNCTIONAL AND ANATOMICAL DEVELOPMENT OF THE CEREBRUM.

By WESLEY MILLS.

HISTOLOGICAL observations in progress on the brains of very young animals will probably show that in all cases of early functional negativity, disappearing later, there is a corresponding immaturity of both the cortex and the white matter beneath it.

A NOTE ON SENSORY NERVE-ENDINGS IN THE EXTRINSIC EYE MUSCLES OF THE RABBIT — ATYPICAL MOTOR-ENDINGS OF RETZIUS.

By G. CARL HUBER.

In his third volume of the "Biologische Untersuchungen" Retzius gives an account of observations made with the Golgi and methylene-blue methods on motor-endings in striated muscle tissue, and here draws attention to an "atypical motor-ending" found in the extrinsic eye muscles of the rabbit. The nerve-endings thus described have been repeatedly observed by me in the course of an investigation, now in progress, on the ending of sensory nerves in the eye muscles of vertebrates. That the "atypical motor-endings" of Retzius are sensory and not motor nerve-endings may, I believe, be argued from the following data: (1) the nerve-endings in question are found mainly in the anterior third of the recti muscles of the eye, while typical motor-endings are found in the middle third; (2) the nerves terminating in this ending present near their termination short internodal segments, branch frequently, and are surrounded by a thin sheath of Henle; agreeing in these respects with sensory nerves in other parts of the body; (3) the ending is found in the connective tissue, immediately outside of the sarcolemma of the muscle fibres between which they terminate, while the motor nerves end under the sarcolemma of the muscle fibres; (4) no other sensory nerve-endings have been found in the extrinsic eye muscles of the rabbit, while in other mammals studied such endings may readily be found.

ON THE PATHS OF ABSORPTION FROM THE PERITONEAL CAVITY.

By LAFAYETTE B. MENDEL.

In a number of experiments regarding the paths by which colored fluids are absorbed from the peritoneal cavity, it was observed under a variety of conditions that in every case the colored substance (indigo-carmine) appeared in the urine considerably earlier than in the lymph flowing from the thoracic duct. The conditions of the experiments were carefully regulated to avoid the deficiencies of previous investigators, and the author is inclined to the blood-vessel theory of absorption, as suggested by Starling.

PRELIMINARY COMMUNICATION ON THE ABSORPTION OF PROTEIDS.

By P. A. LEVENE and I. LEVIN.

IT has always been the accepted theory, though based on imperfect experimental testimony, that proteid material is absorbed from the gastro-intestinal tract by the blood system. Recently, however, Asher and Barbèra have subjected the question to a new trial, and obtained results that lead them to conclusions contradictory to the generally accepted view.

The great difficulty of solving the problem with exactness lies in the fact that any injected proteid once past the barrier of the intestinal wall cannot be distinguished from the tissue proteids. It was, therefore, the aim of the authors to study the path of absorption of iodoproteids, as the latter could easily be identified in any tissue or organ. A ten per cent solution of iodoproteid was injected into a previously cleaned and ligated loop of the colon and rectum, and the proteid was searched for in the lymph of the thoracic duct. The results of three experiments are negative. Hence these results tend to corroborate the old theory and contradict the view taken by Asher and Barbèra.

The questions the authors are now endeavoring to answer are: what component of the blood (leucocytes or plasma) carries the proteids from the intestinal wall; and in what organ or organs is the iodoproteid decomposed before being assimilated (if it is assimilated at all).

THE BEHAVIOR OF INULIN IN THE GASTRO-INTESTINAL TRACT.

By R. H. CHITTENDEN.

A SERIES of experiments on the behavior of inulin in the gastro-intestinal tract carried out in the writer's laboratory by Arthur B. Siviter has led to the following conclusions.

The ordinary amylolytic enzymes, such as the ptyalin of saliva, the amylopsin of pancreatic juice, vegetable diastase, and "Taka" diastase are without action on inulin.

Dilute hydrochloric acid (0.05 — 0.2 per cent) at 40° C. inverts inulin to levulose. Combined hydrochloric acid (combined with proteids) likewise inverts inulin to levulose, but more slowly than corresponding strengths of *free* acid.

Organic acids, such as oxalic, lactic, and salicylic, also transform inulin to levulose.

The value of inulin as a food is probably due to the fact that the hydrochloric acid of the gastric juice may invert inulin to levulose, the latter being absorbed and utilized in increasing the store of glycogen in the liver.

THE PRODUCTION OF FLUORESCENT PIGMENT BY BACTERIA.

BY EDWIN O. JORDAN.

THE outcome of experiments made with six different cultures of "fluorescent bacteria" was as follows:

1. The presence of both phosphorus and sulphur is essential to the formation of the fluorescent pigment.

2. The nature of the base associated with the phosphorus and sulphur is not important.

3. The conclusions that may be drawn regarding the dependence of the fluorescent "function" upon the molecular constitution of the ammonium salts may be best appreciated through an examination of the constitutional formulæ of the organic acids whose salts were employed. The list is arranged as far as possible in order of fluorescigenic value.

1. Asparagin . .	$COOH . CH_2 . CH(NH_2) . CONH_2$.
2. Succinic acid .	$COOH . CH_2 . CH_2 . COOH$.
3. Lactic acid . .	$CH_3 . CHOH . COOH$.
4. Citric acid . .	$COOH . C(OH) . (CH_2COOH)_2$.
5. Tartaric acid .	$COOH . CHOH . CHOH . COOH$.
6. Uric acid . . .	$NH . CO . NH . COC = C . NH . CO . NH$.
7. Acetic acid . .	$CH_3 . COOH$.
8. Oxalic acid . .	$COOH . COOH$.
9. Formic acid . .	$H . COOH$.

The fluorescence is not intimately bound up with the bibasicity of the acid, and the existence in the molecule of at least two groups of CH_2 (as Lepierre asserts). The difference between acetic acid on the one hand and oxalic and formic acids on the other is certainly significant, but that neither the carboxyl (COOH) nor the methylene (CH_2) grouping is essential to pigment production is shown by the availability of urate. The difference between tartrate and succinate, as well as that between formate and acetate, does, however, clearly indicate that, other things being equal, the presence of the methyl or methylene group is coincident with superior nutritive value and fluorescigenic power.

4. The presence of acid in the medium not merely conceals the existence of the substance to which the color is due, but interferes with those vital activities of the bacilli which, in an alkaline solution, lead to the production of that substance.

5. Diffuse daylight is unfavorable to pigment production.

6. If chemical substances that prove, when in certain proportions, favorable to growth and to the production of pigment be present in excess of a certain quantity, the production of pigment will be checked, although growth may be more abundant than before.

A NEW BRAIN MICROTOME.

By C. F. HODGE (with H. H. GODDARD).

In the new microtome the upper surface of the knife is level, and, being hollow ground, the knife thus carries the pool of liquid into which the section floats. This does away with the dripping bottle. The knife blade proper being only 2½ inches wide the surface is widened to 8 inches by a simple pan of zinc screwed to the back of the blade and sealed on with melted paraffine. The pool of liquid on the blade is about ¼ inch deep. In order to give a sufficient obliquity of cut a long blade is required. The blade in question is 36 inches in length, and since it would be difficult to support such a blade at one end and would require a very heavy sledge to hold it firmly, it was proposed (Mr. Goddard's contribution of special value to the problem) to support the knife firmly at both ends. This has made it necessary to have the specimen move against the edge of the stationary knife. No difficulty was encountered, however, on this account, the brain being mounted on a case which slides upon a heavy iron track running obliquely underneath the knife. This case, a hollow box of cast iron, slides smoothly in an outer case of similar construction, and the micrometer screw is fastened to the bottom of the outer case and nutted through the bottom of the inner case so that the inner case may be moved up or down within the outer. The outer case is provided with projections which support it on the track, and the whole is moved from one end of the track to the other easily and smoothly by means of a rack and pinion. A single notch on the screw head gives a section 12½ micra thick.

The knife is made of a soft iron body into which an edge of very hard razor steel is welded. This has obviated the warping and twisting attendant on high tempering of solid steel blades and has resulted in a razor with a perfectly true edge. The framework of the microtome is made of cast iron, rigidly braced. The cost of constructing the machine was about $150.00.

SOME FACTS RELATING TO THE MELANINS. By R. H. CHITTENDEN.
See this Journal, vol. ii, p. 291.

ON THE CAUSES OF THE ORDERLY PROGRESS OF THE PERISTALTIC MOVEMENTS IN THE ŒSOPHAGUS. By S. J. MELTZER.
See this Journal, vol. ii, p. 266.

EXPERIMENTS ON THE MAMMALIAN HEART. By W. T. PORTER.
See this Journal, vol. ii, p. 127.

A CONVENIENT FORM OF NON-POLARIZABLE ELECTRODE. By W. H. HOWELL.

NEW LABORATORY APPARATUS. By E. W. SCRIPTURE.

AN IMPROVED FORM OF ELLIS'S PISTON RECORDER. By W. P. LOMBARD.

A SIMPLE ONCOMETER. By FREDERIC S. LEE.

DEMONSTRATION: METHYLENE BLUE PREPARATION OF SENSORY NERVE-ENDINGS IN TENDON — GOLGI'S TENDON CORPUSCLES. By G. C. HUBER.

A NEW RESPIRATION APPARATUS. By FREDERIC S. LEE.

DEMONSTRATION: THE CO-ORDINATION OF THE VENTRICLES. By W. T. PORTER.

EXHIBITION OF INSTRUMENTS FOR THE STUDY OF MOVEMENT AND FATIGUE. By J. McK. CATTELL.

THE PHYSIOLOGICAL BASIS OF MENTAL LIFE. By H. MÜNSTERBERG.

CONFUSION OF TASTES AND ODORS. By G. T. W. PATRICK.

METHODS OF DEMONSTRATING THE PHYSIOLOGY AND PSYCHOLOGY OF COLOR. By E. W. SCRIPTURE.

ON THE FLICKER PHOTOMETER. By O. N. ROOD.

A CONVENIENT FORM OF SPHYGMOGRAPH. By R. H. CHITTENDEN.

A CHEMICO-PHYSIOLOGICAL STUDY OF CERTAIN DERIVATIVES OF THE PROTEIDS. By R. H. CHITTENDEN.

See this Journal, vol. ii, p. 142.

EXPERIMENTS ON THE MOLECULAR CONCENTRATION AND ELECTRICAL CONDUCTIVITY OF CERTAIN ANIMAL LIQUIDS. By G. N. STEWART.

THE CHOLESTERIN-ESTERS OF BIRDS' BLOOD. By E. W. BROWN (presented by L. B. MENDEL).

See this Journal, vol. ii. p. 306.

INFLUENCE OF MASSAGE UPON THE NUMBER OF BLOOD GLOBULES IN THE CIRCULATING BLOOD. By J. K. MITCHELL (read by H. P. BOWDITCH).

AN INSTRUMENT FOR MEASURING MUSCULAR TONICITY IN MAN. By L. J. J. MUSKENS.

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